Targeting of Cancer Stem Cells Overcomes Platinum Chemoresistance

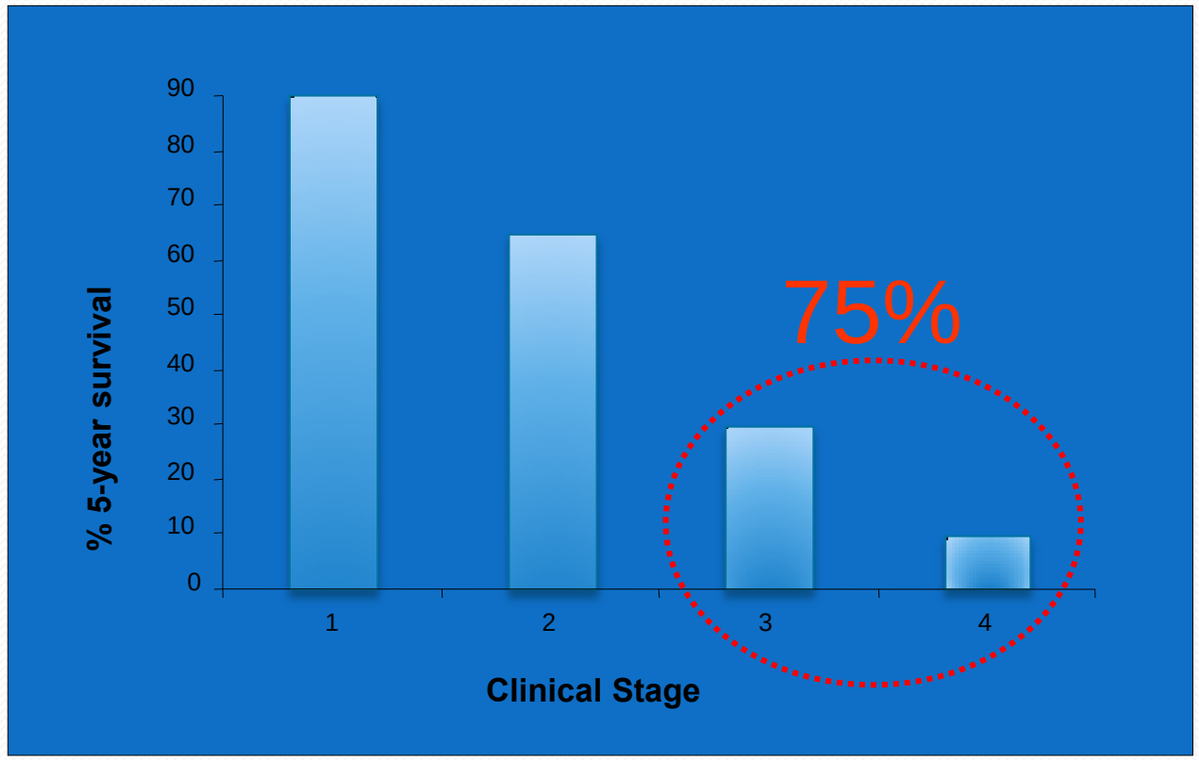
Daniela Dinulescu, PhD
Assistant Professor
Harvard Medical School

BRIGHAM AND WOMEN'S HOSPITAL

HARVARD MEDICAL SCHOOL

Ovarian Cancer Demographics

- The most deadly gynecologic malignancy.
- Late diagnosis = poor survival.
- Overcoming resistance to platinum chemotherapy critical for long-term remission.

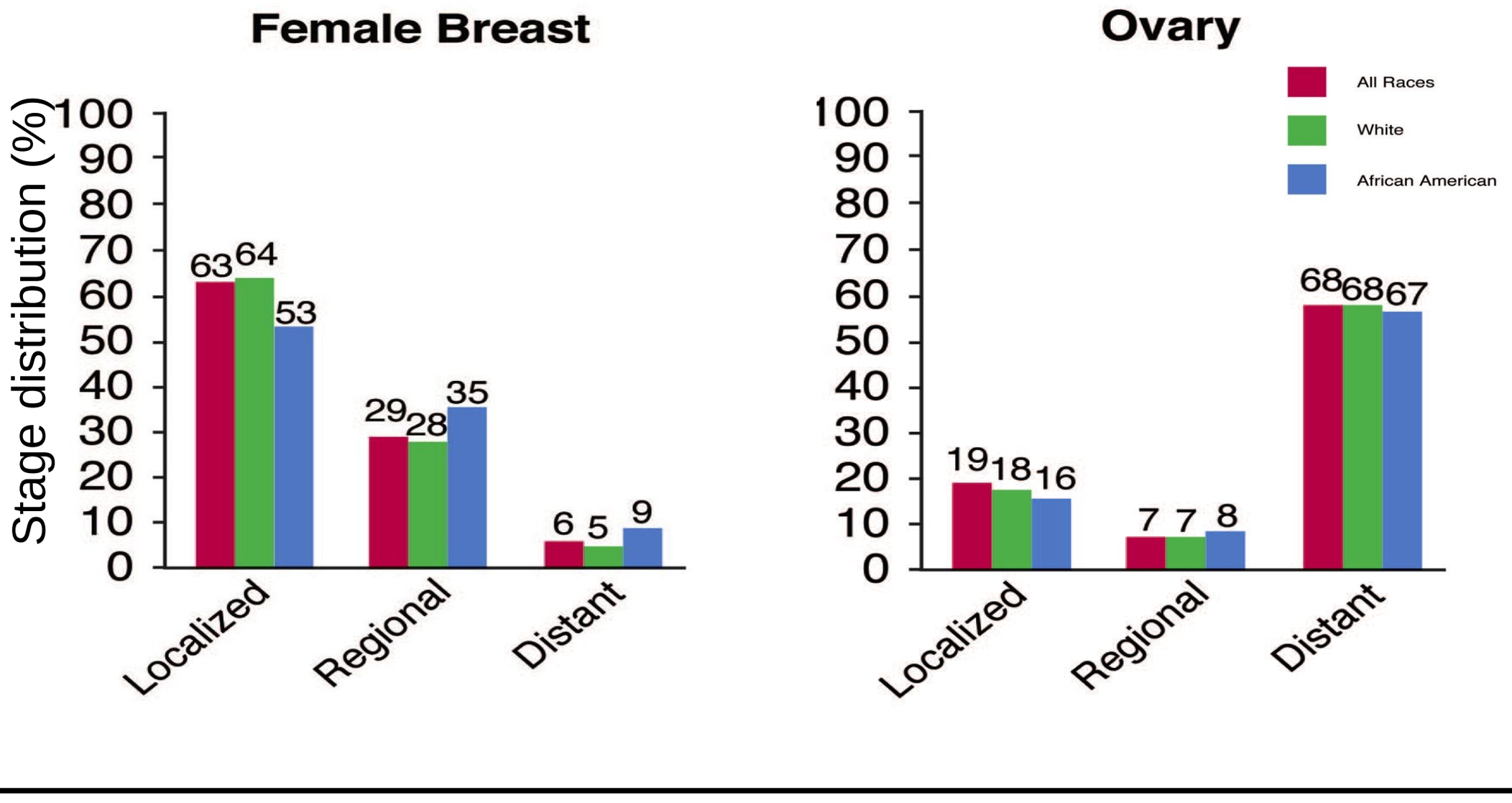


- **Major factors contributing to the high mortality rate:**

-late stage diagnosis

-absence of a screening test for early diagnosis

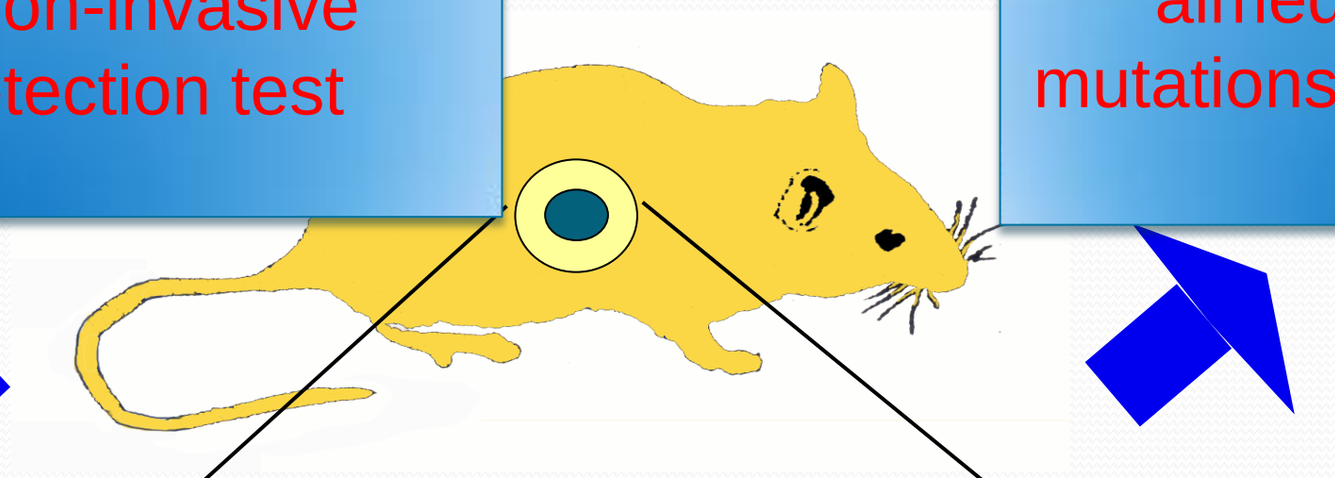
-limited treatment options for patients resistant to platinum therapy.



Research Goals of Our Laboratory

EARLY DETECTION
Identify plasma/tumor biomarkers to develop a non-invasive blood/urine detection test

Improve PLATINUM CHEMORESISTANCE aimed at key genetic mutations, cancer stem cells



Development and Validation of
Genetically-engineered
and Patient-derived Animal Models

EARLY DETECTION
Develop IMAGING tools to detect early disease and guide treatment

PREVENTION
Develop vaccines
Identify benign precursors
Chemopreventive agents

Role of Ovarian CSCs in Platinum Chemoresistance

- The majority of patients respond to surgical cytoreduction and chemotherapeutic regiments that include platinum and paclitaxel.

- While standard treatment can result in clinical remission independent of disease stage, more than 2/3 of patients will relapse and develop increased resistance to platinum.

- It is therefore essential to target mechanisms regulating intrinsic and acquired resistance.

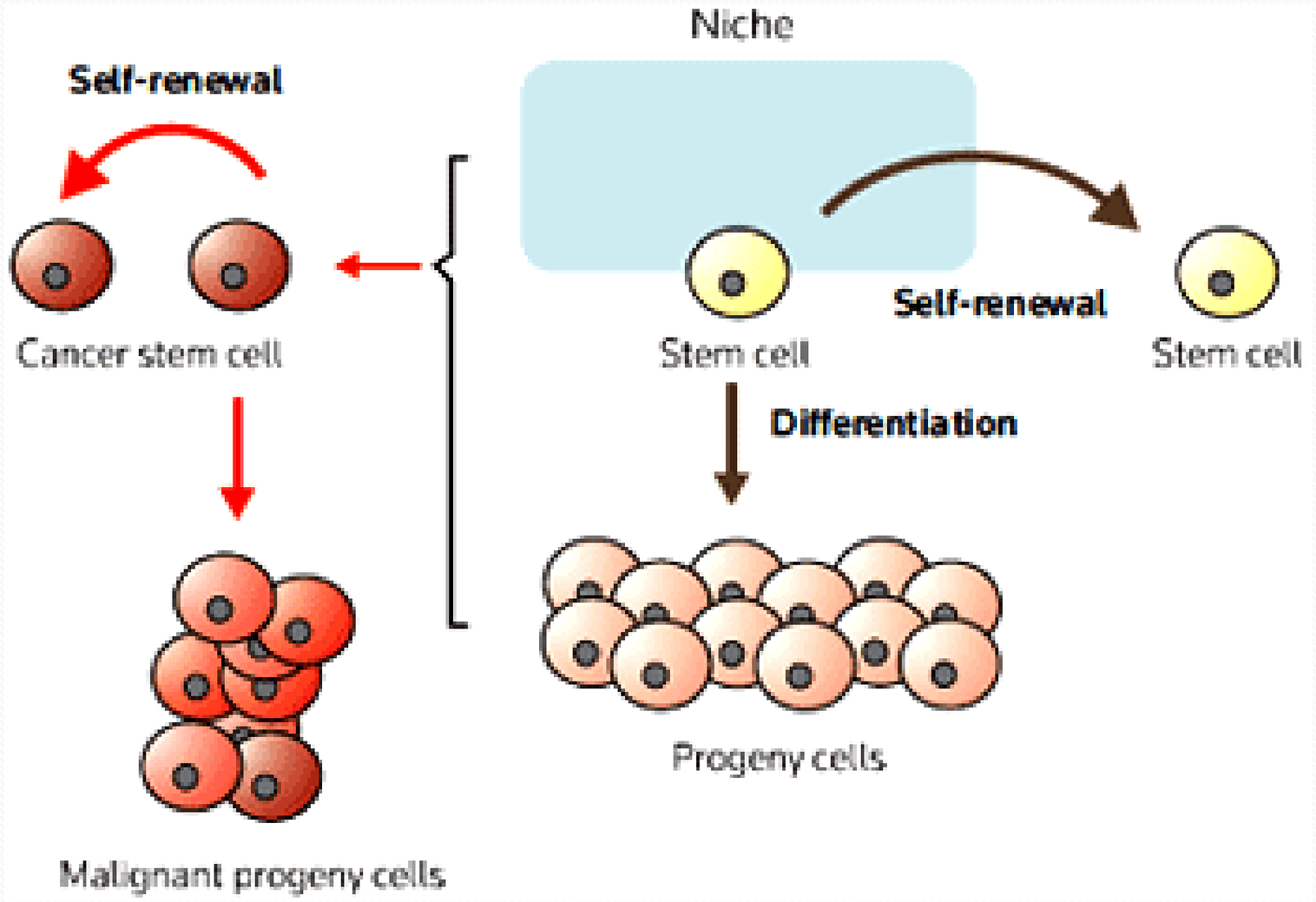
- Cancer Stem Cells (CSC) = Increased cancer initiating capacity.
  - Oncogenesis and Cancer Maintenance
  - Chemoresistance to therapy: ABC Multi-drug transporters
  - Express distinctive cell markers allowing for consistent isolation

Key Properties of CSCs

- Propensity to indefinitely divide, involved in cancer invasion and metastasis.

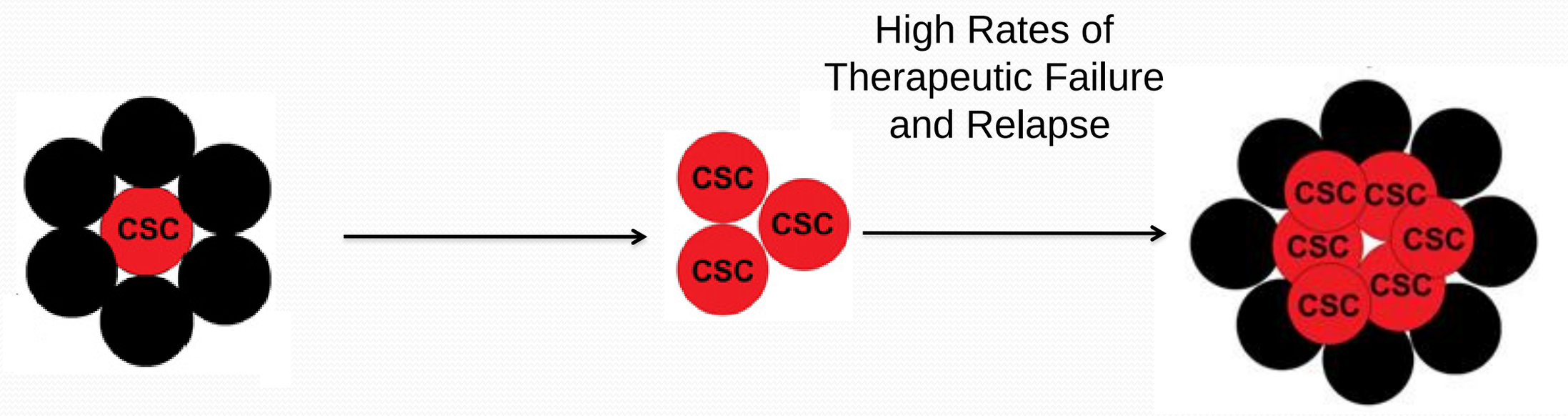
- Major contributor to platinum resistance.

- Ability to regenerate a heterogenous tumor.



(Romano 2009)

Chemoresistance and Cancer Stem Cells



Platinum Sensitive Tumor

Tumor Exposure
to Platinum Therapy Leads
to Steady Accumulation of
Drug-Resistant CSCs

High Rates of
Therapeutic Failure
and Relapse

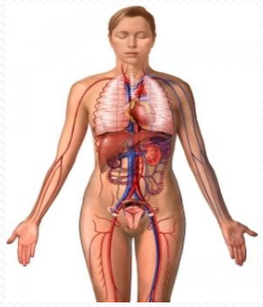
Relapse After Platinum
Therapy is Increasingly
Platinum Resistant

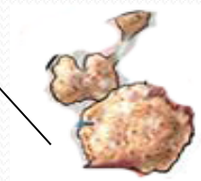
CSCs Studies in Tumor Models and Patient Samples

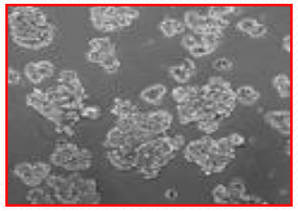


Ovarian cancer models

Patient samples



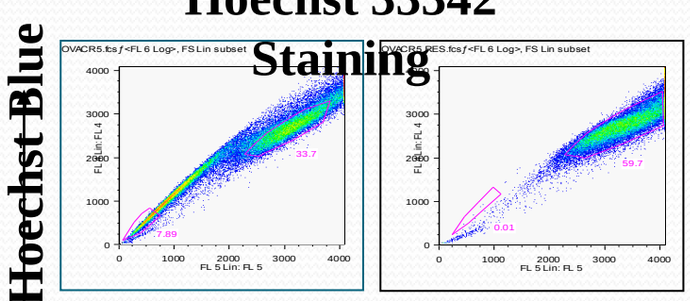




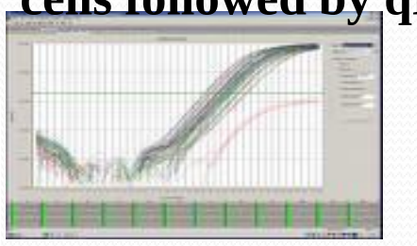


Dako-Cytomation MoFlo Cell Sorter

Hoechst 33342 Staining



RNA extraction from sorted SP and NSP cells followed by qRT-PCR analysis





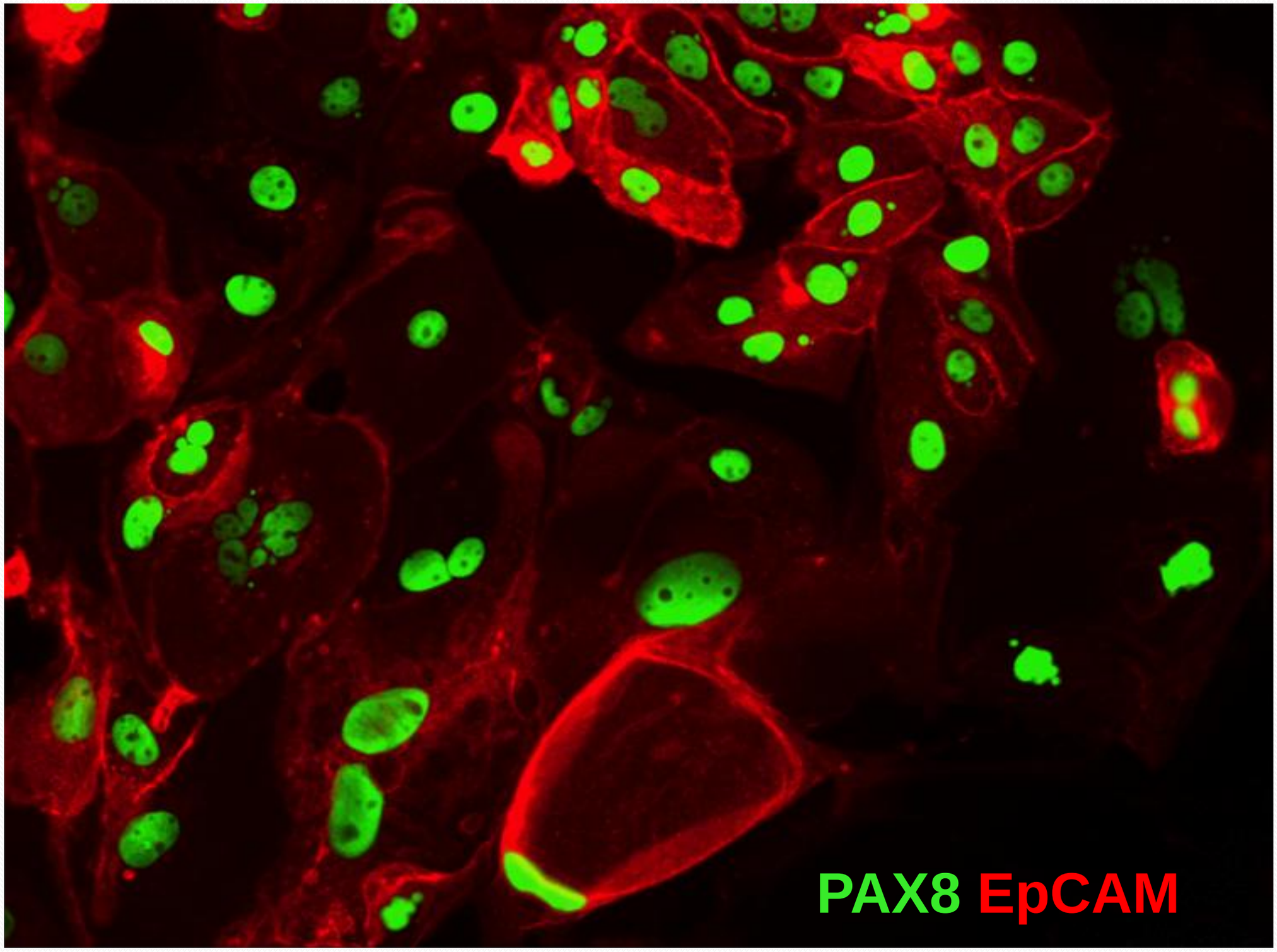




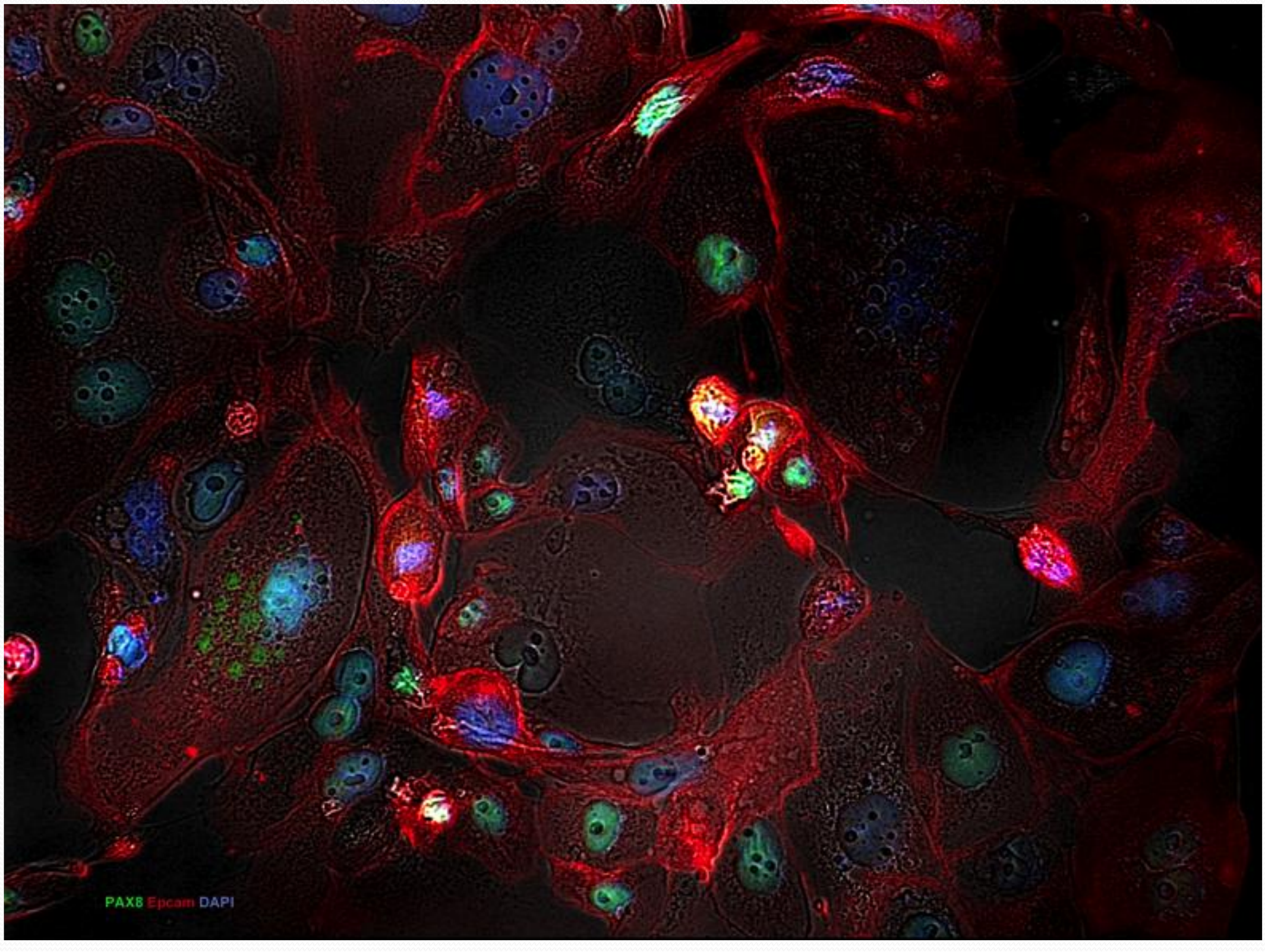
Applied Biosystem 7300 Real time PCR system

***Stem Cell PCR Array**
***Cancer Drug Resistance and Metabolism PCR Array**
***Extracellular Matrix and Adhesion Molecules PCR Array**
***Cell Surface Markers PCR Array**

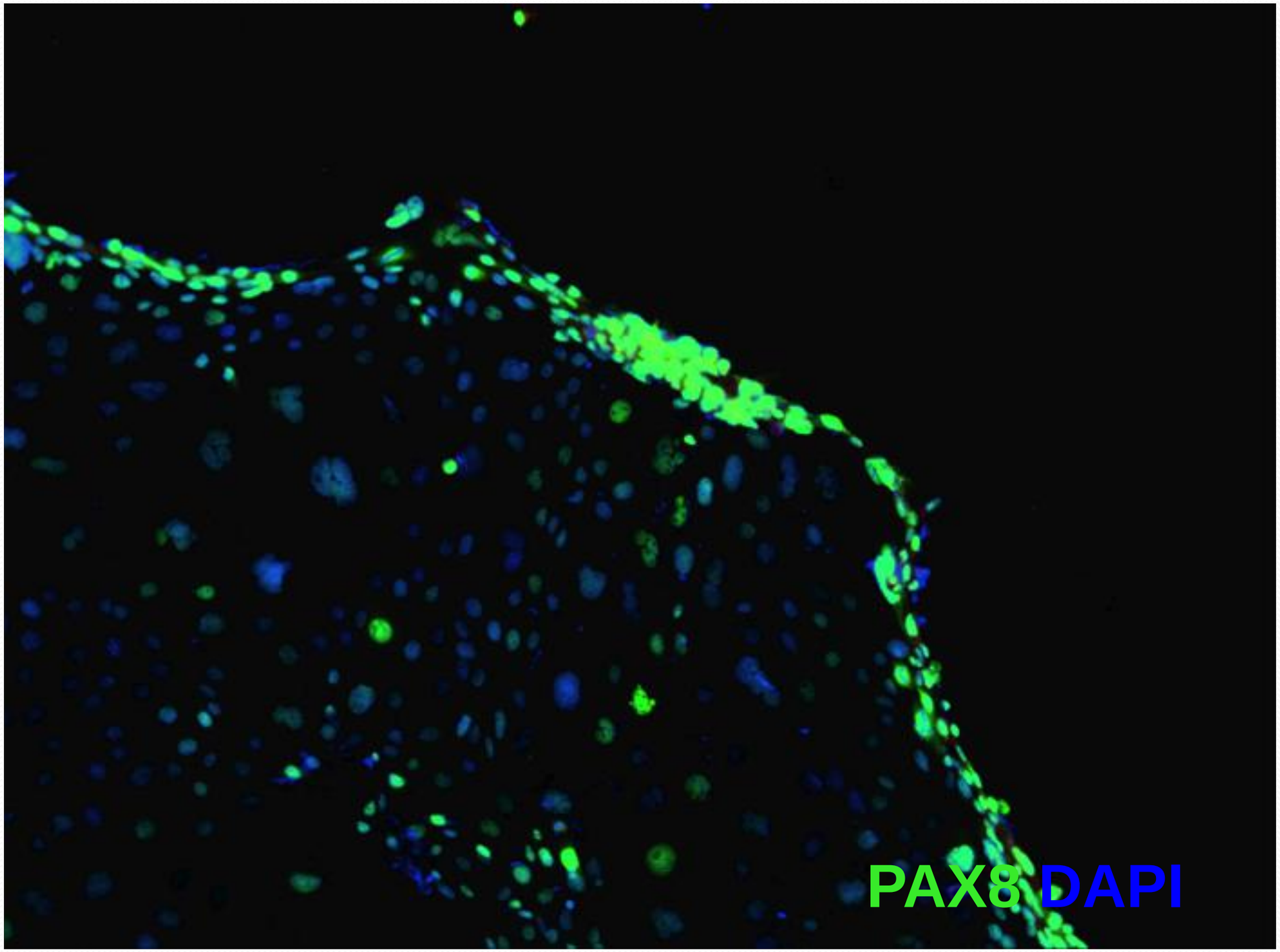
ASC117-Patient Ascites



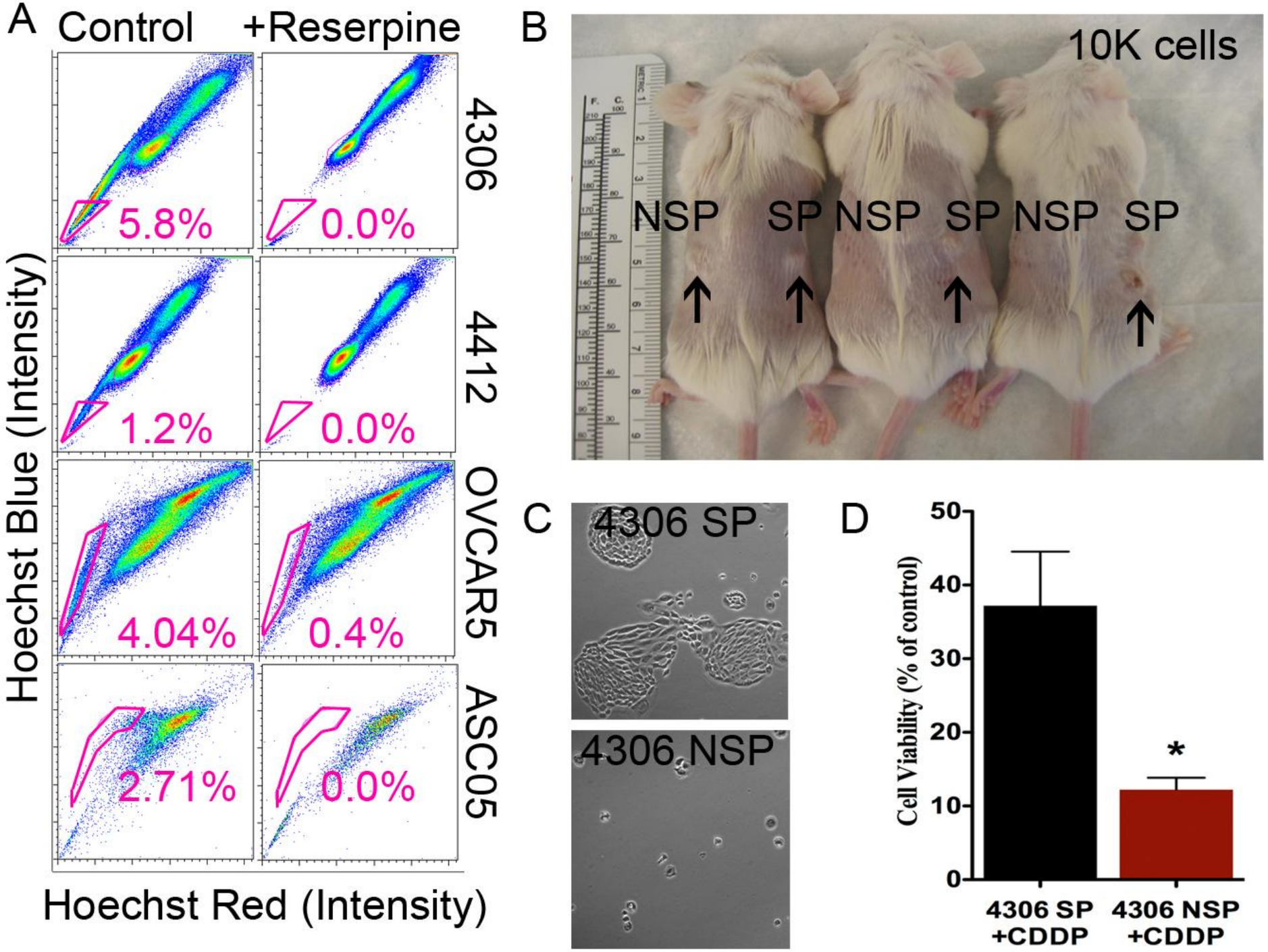
ASC 120
Overlay with Phase image



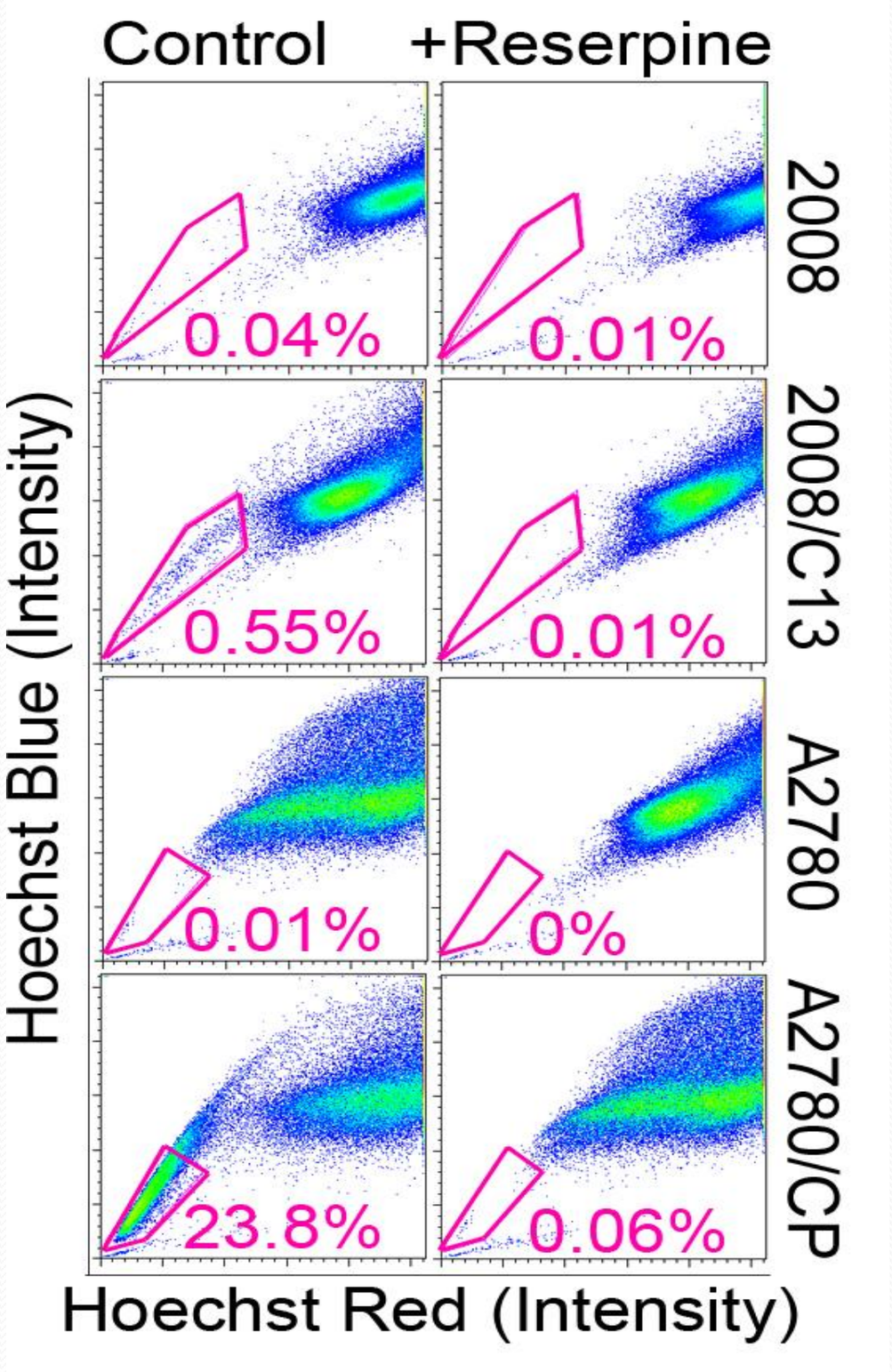
mFTSEC 919



Ovarian CSCs Are More Resistant to Platinum



Ovarian CSCs Are Enriched in Platinum Resistant Lines



Cisplatin (CDDP) sensitive lines

2008

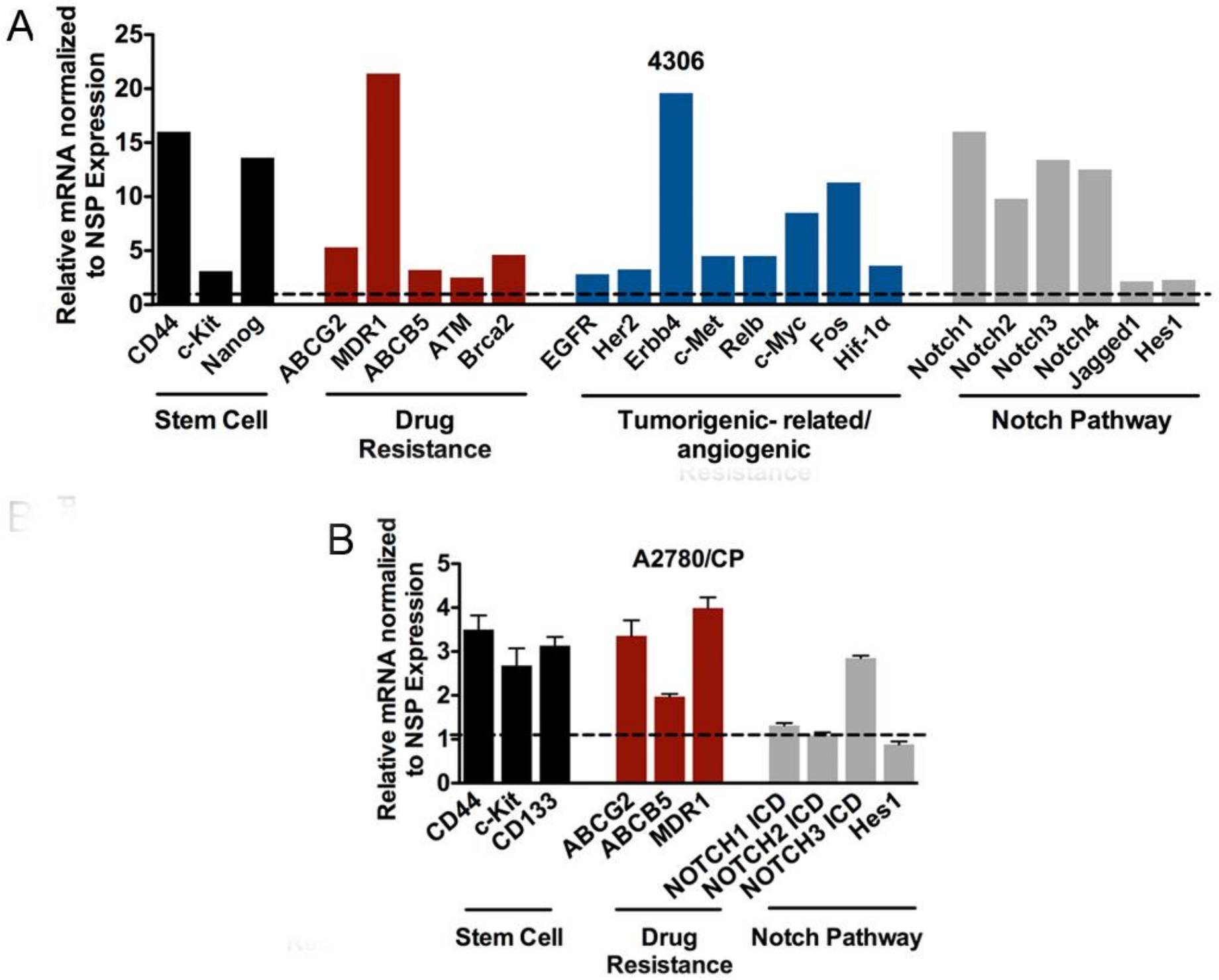
A2780

CDDP resistant lines

2008/C13

A2780/CP

Ovarian CSCs Molecular Profile (Array Analysis)



CSC Markers Are Conserved Across Cancer Types

Ovarian	Breast	Colon	Glioma	Liver	Lung	Melanoma	Pancreatic	Prostate
ALDH1	ALDH1	ALDH1			ALDH1	ALDH1	ALDH1	ALDH1
CD133	CD133	CD133	CD133	CD133	CD133	CD133	CD133	CD133
CD44	CD44	CD44		CD44			CD44	CD44
CD117 (c-Kit)					CD117			
CD24	CD24	CD24		CD24			CD24	
ABCG2					ABCG2		ABCG2	
ABCB5		ABCB5				ABCB5		
MDR1								
	CD90		CD90	CD90	CD90			
		CD166						CD166

Key Ovarian CSC Markers

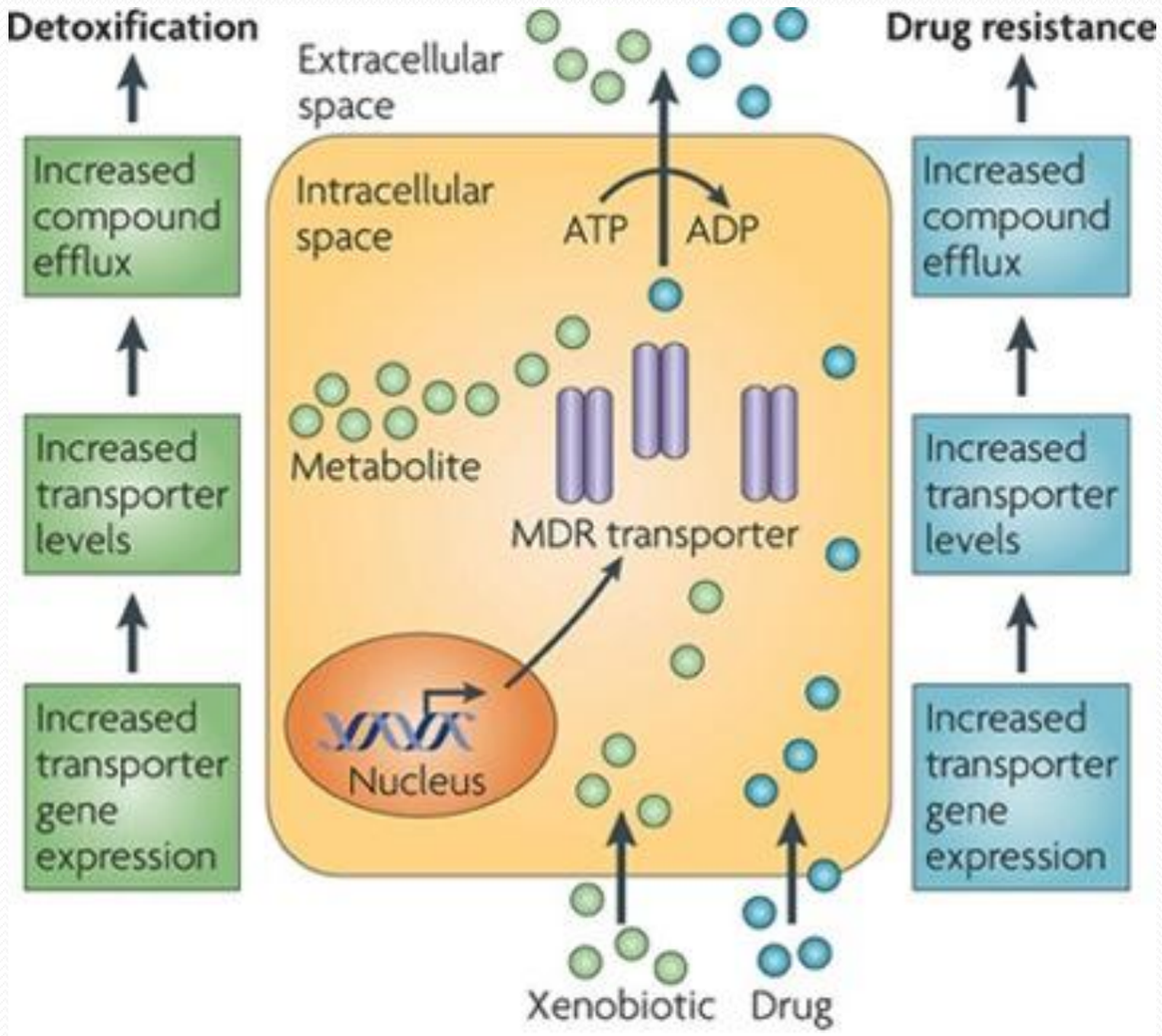
ALDH1

•Overexpression is correlated with highly aggressive, chemoresistant tumors.

CD133

•CD133 overexpression is associated with increased expression of ALDH1 and CD44 in CSCs.

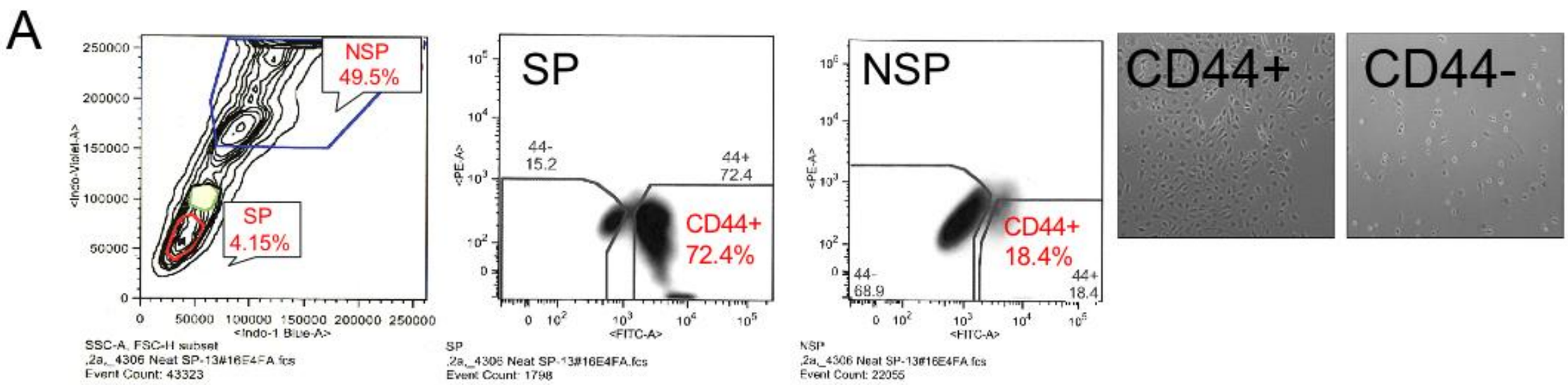
•Overexpression correlated with poor outcome.

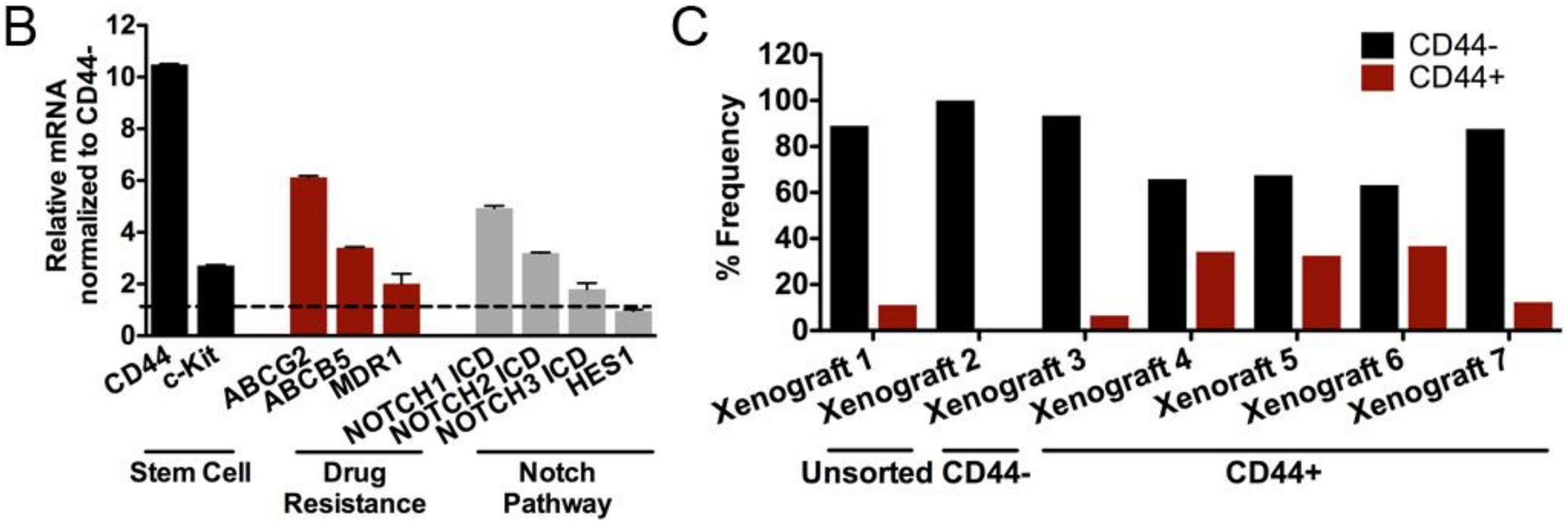


ABC Transporters

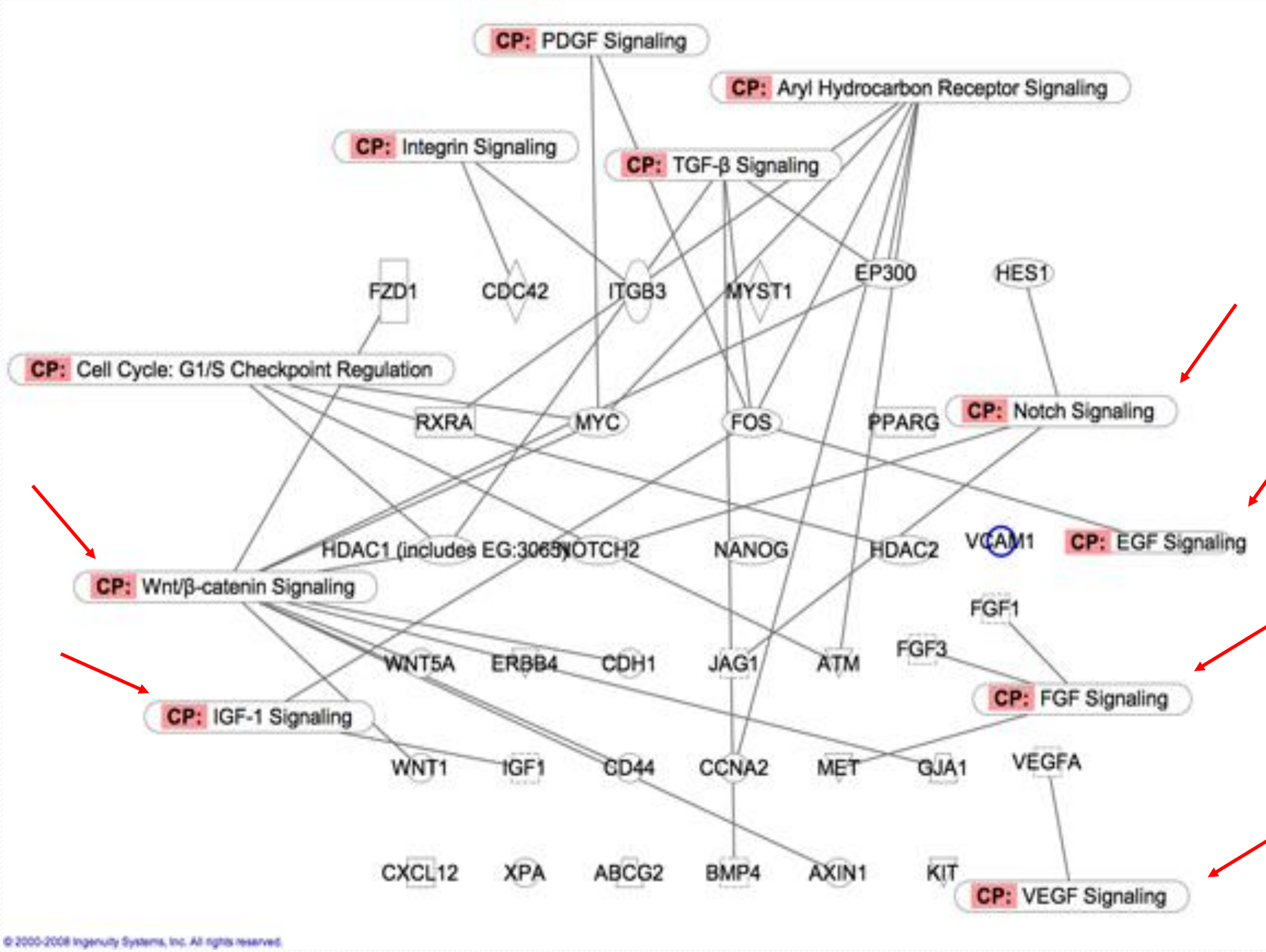
ABC Transporter	Efflux Function
ABCB1 (MDR1, P-gp)	Paclitaxel, doxorubicin, vinblastine
ABCC1 (MRP1)	Cisplatin, doxorubicin, daunorubicin, vincristine, etoposide, colchicine, camptothecins, methotrexate
ABCG2	Topotecan, imatinib, methotrexate, doxorubicin, Hoechst dye 33342

CD44 is Enriched in Ovarian CSCs





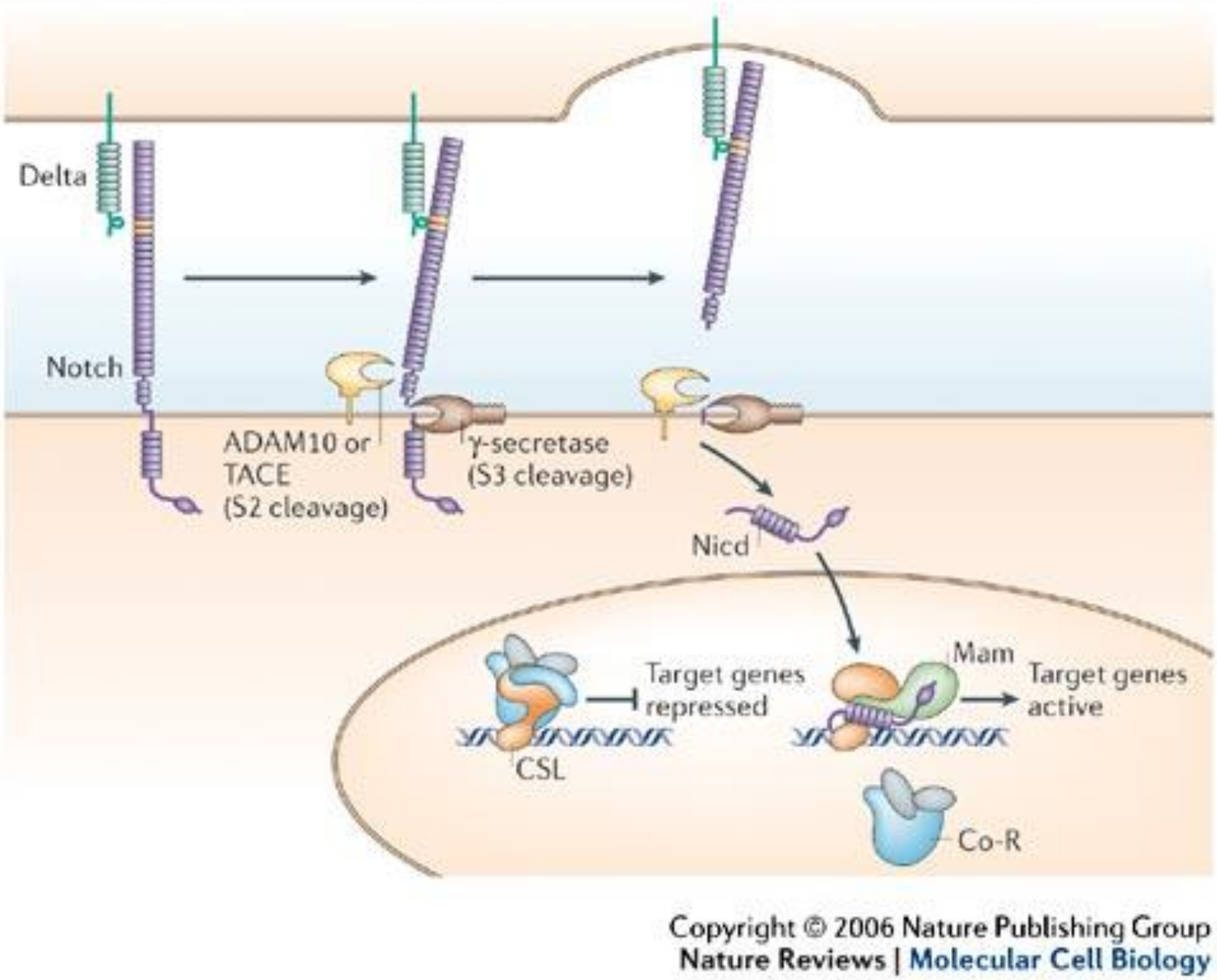
Identification of Key Ovarian CSCs Pathways



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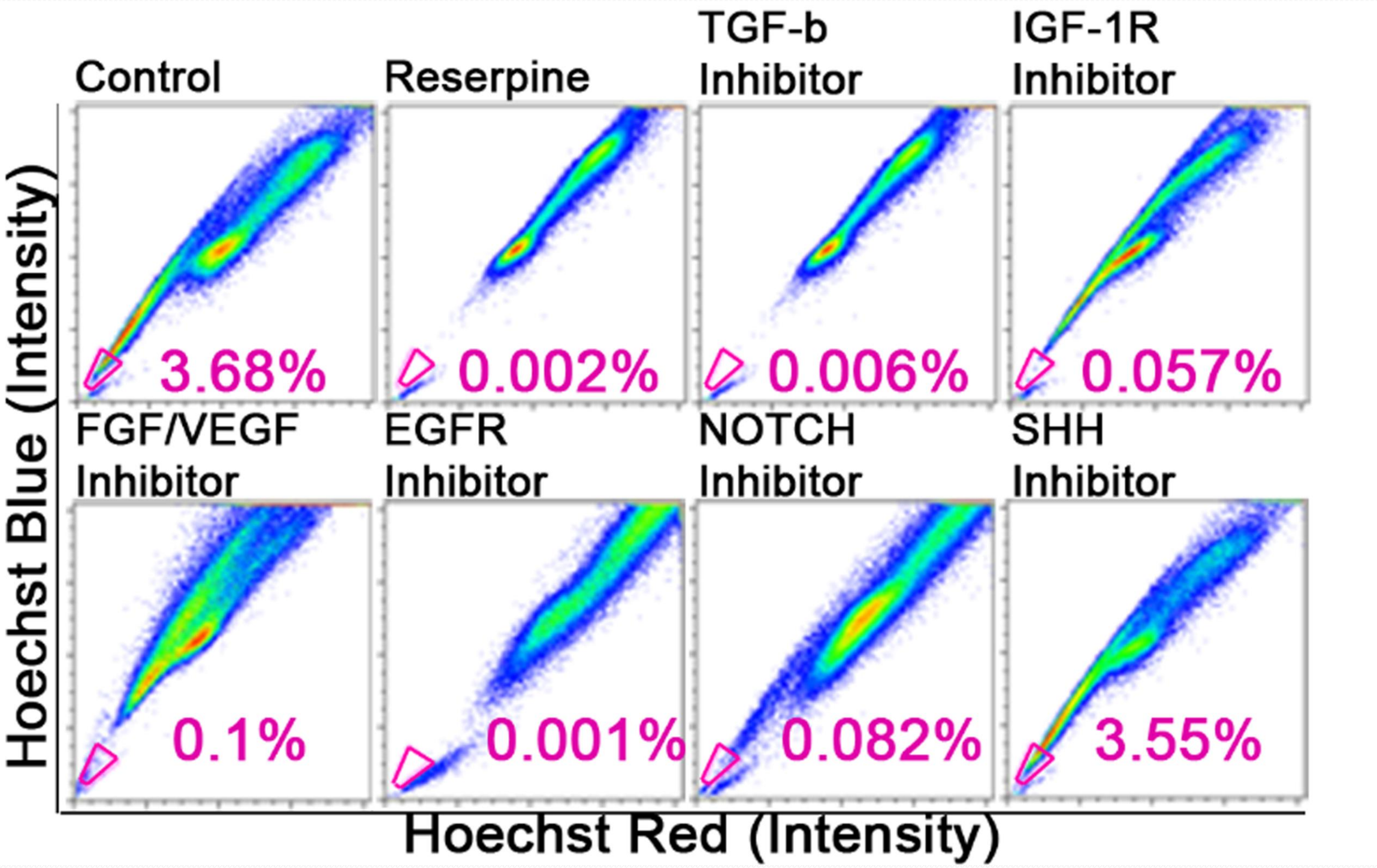
Notch Signaling Pathway

- Active in normal stem cells.
- Implicated in cell fate determination, survival, proliferation.
- Increased Notch activity detected in 22% of ovarian HGSC tumors. (TCGA).

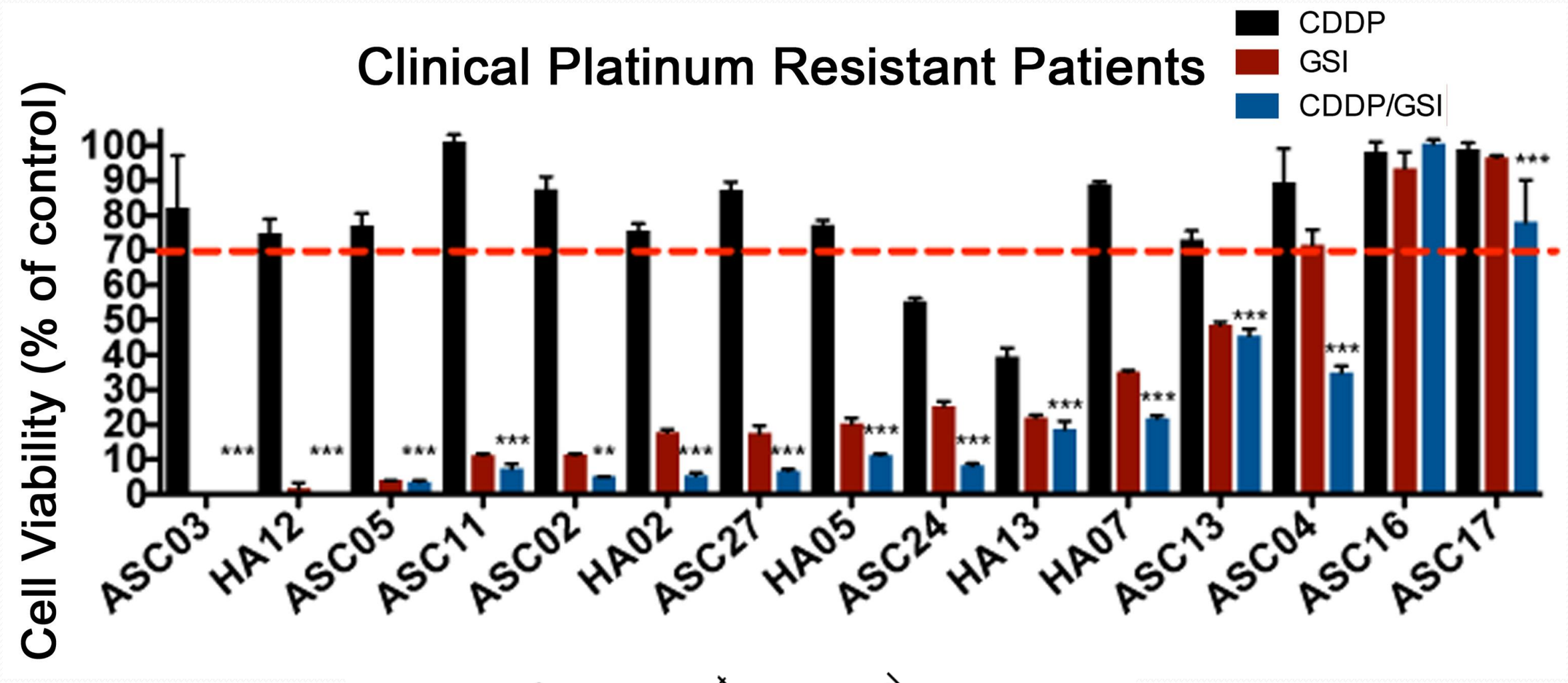


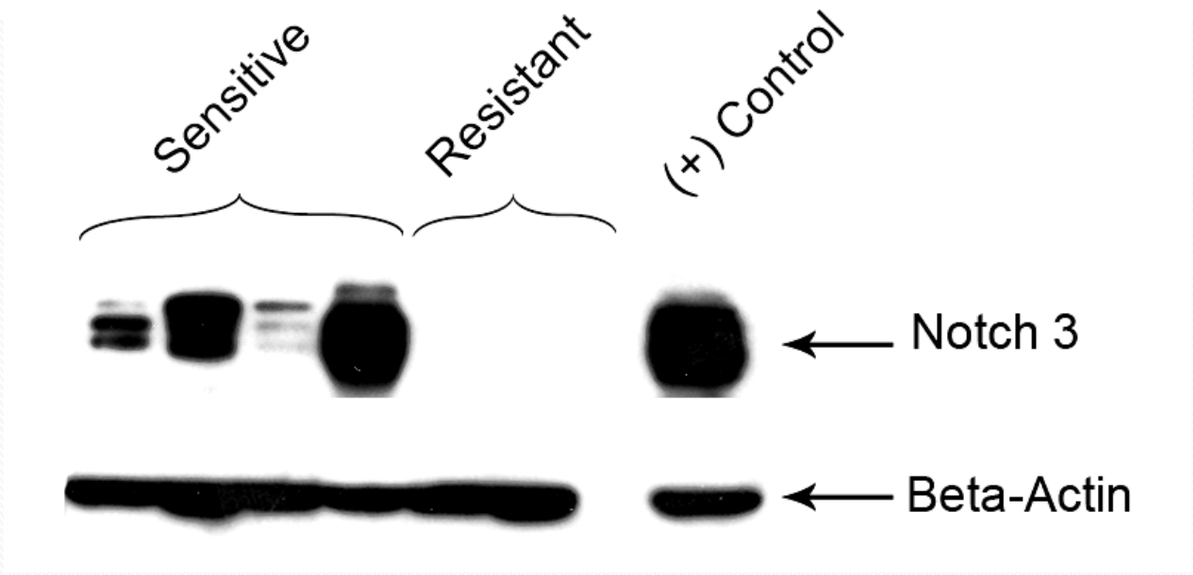
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Key Pathways for Ovarian CSCs Maintenance

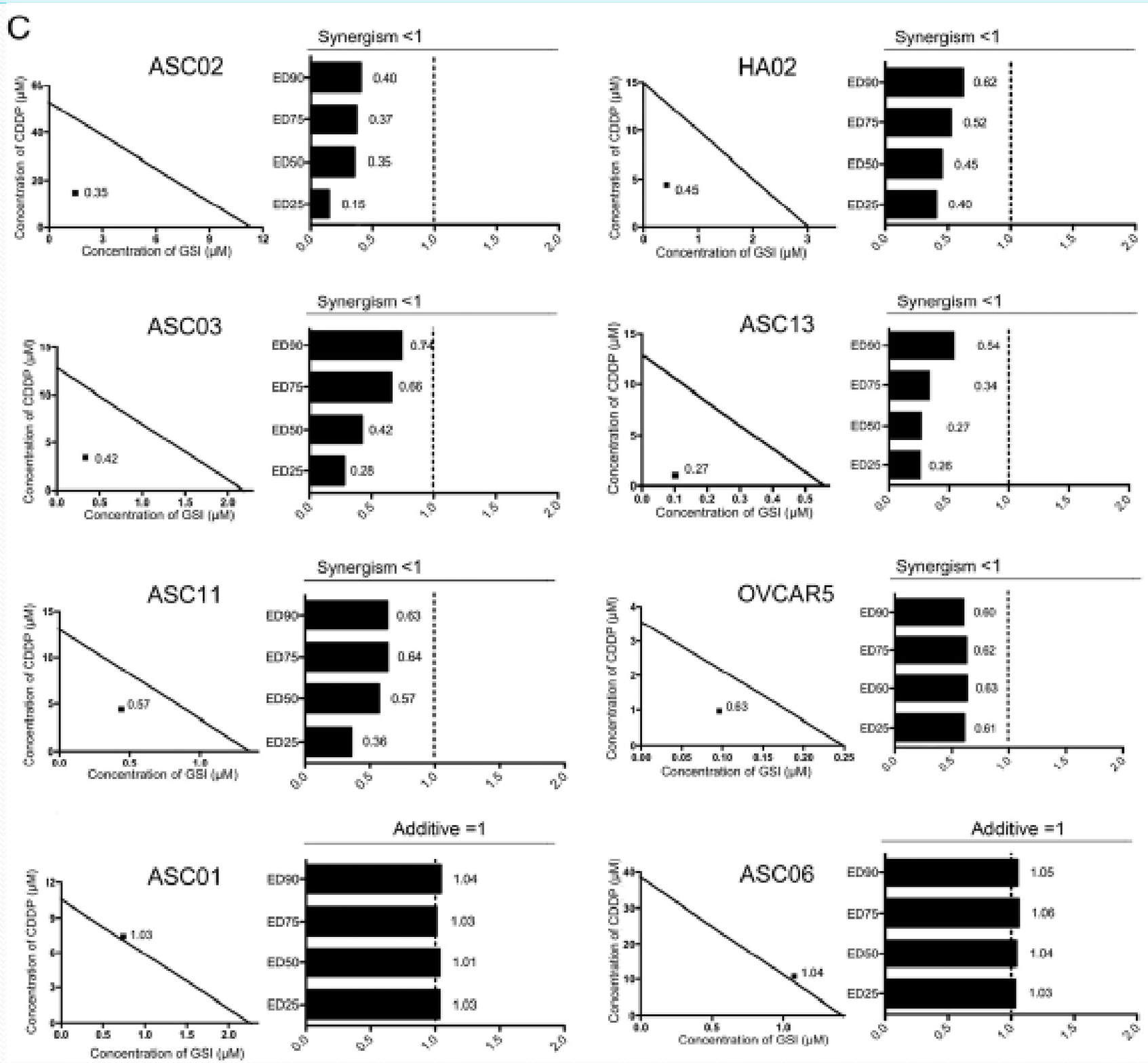


Notch Inhibitors Resensitize Patient Samples to Platinum Therapy

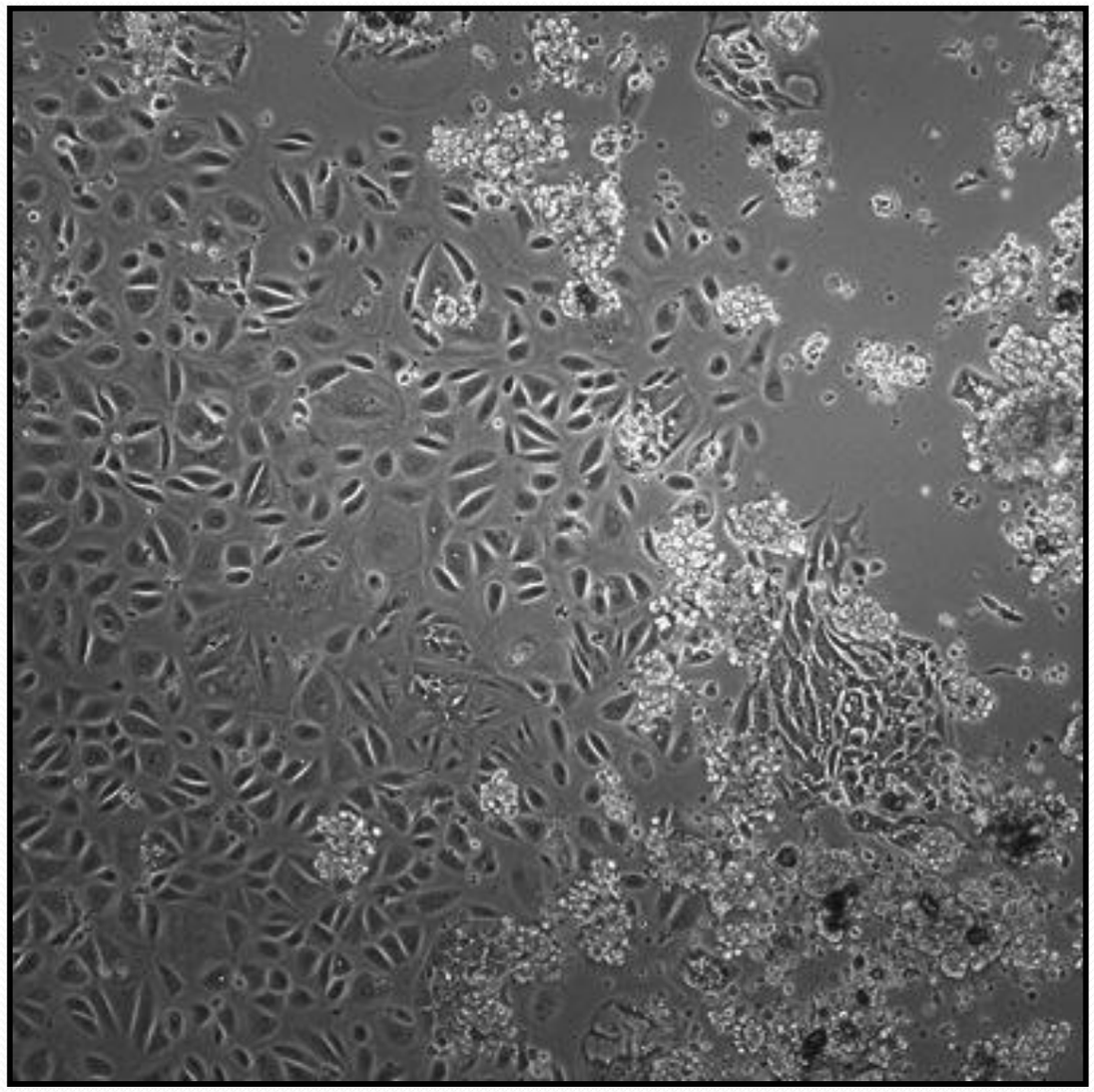




Isobologram Analysis Shows Synergy Between Platinum and Notch Inhibitors

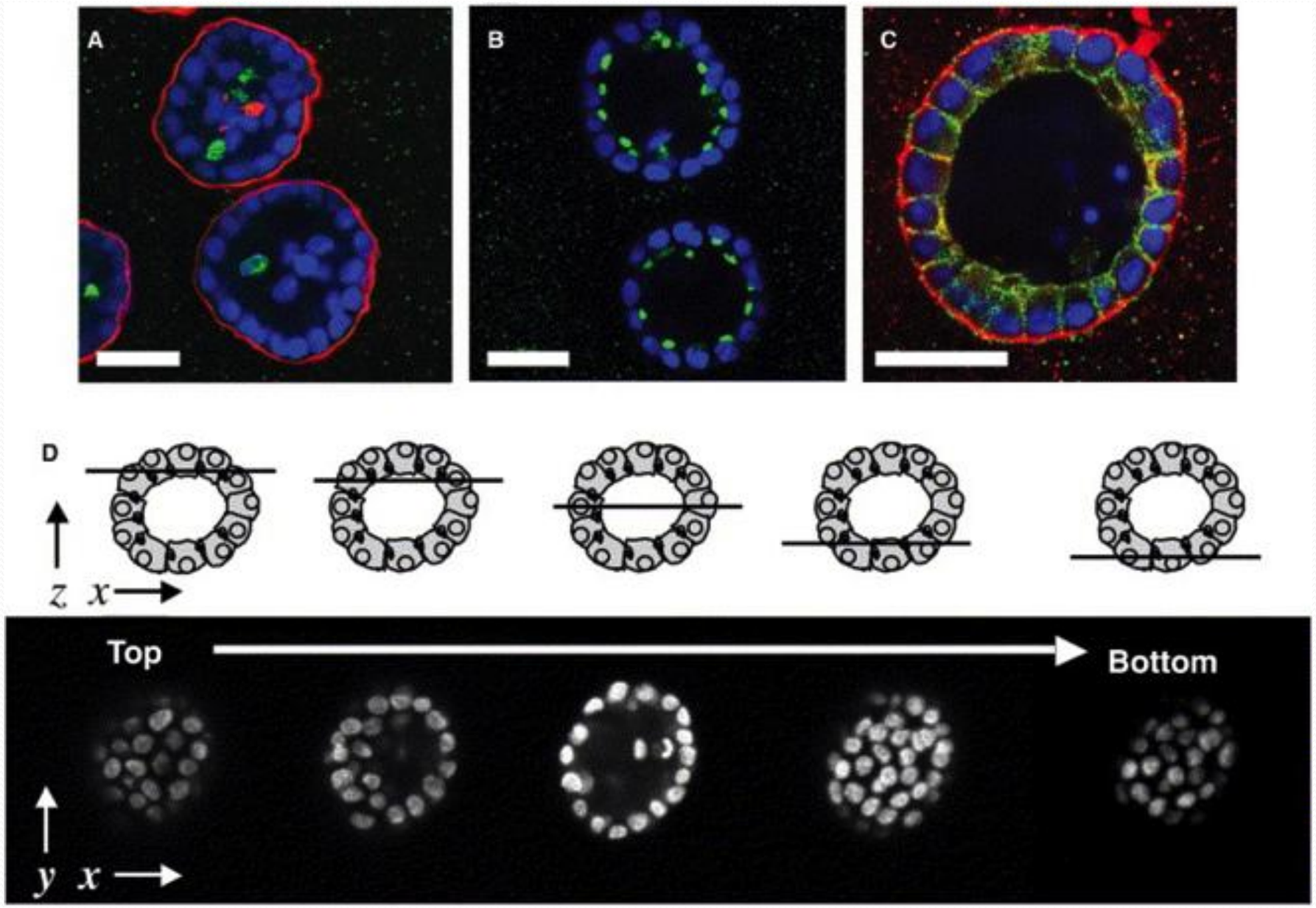


Culturing 3D-Spheroids From Patient Ascites





Immunostaining of 3D-Spheroids

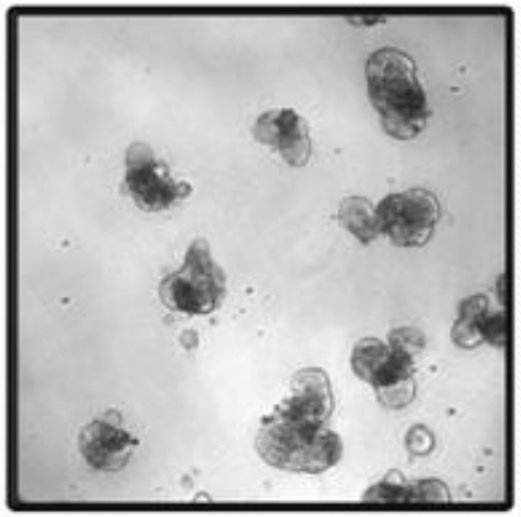


A) MCF-10A cells in matrigel 8 days old. Immunostained activated caspase 3 (green) for apoptosis detection, laminin V (red), DAPI (blue). B) Golgi (GM130) green stain. C) Phospho-ERM (green).

Jayanta Debnath, Sentil K. Muthuswamy, and Joan S. Brugge. ”Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures." Methods 5 Feb. (2003): 256-268. Print.

Platinum Treatment of 3D-Spheroids

Cisplatin 20μM



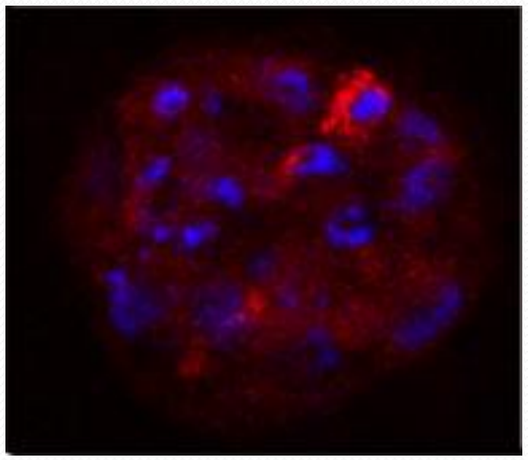
No Treatment



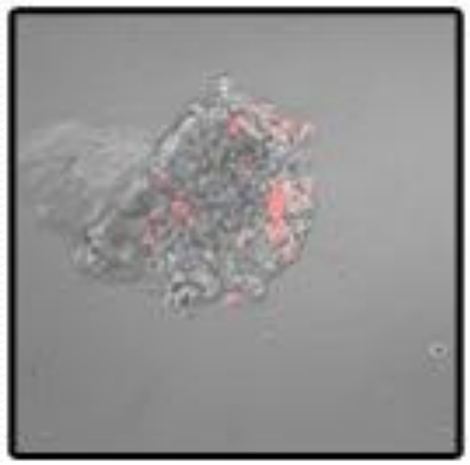
2.5μM Cisplatin, 0.25μM GSI



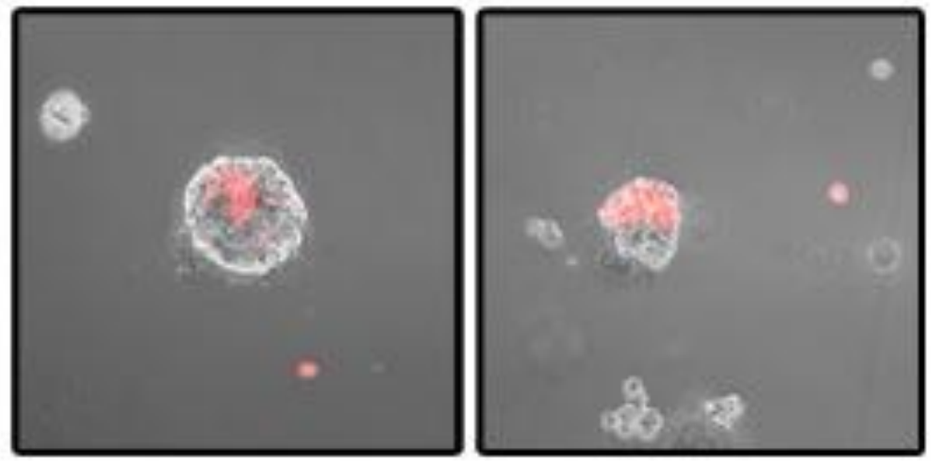
5μM Cisplatin, 0.5μM GSI



10μM Cisplatin, 1μM GSI



20μM Cisplatin, 2μM GSI

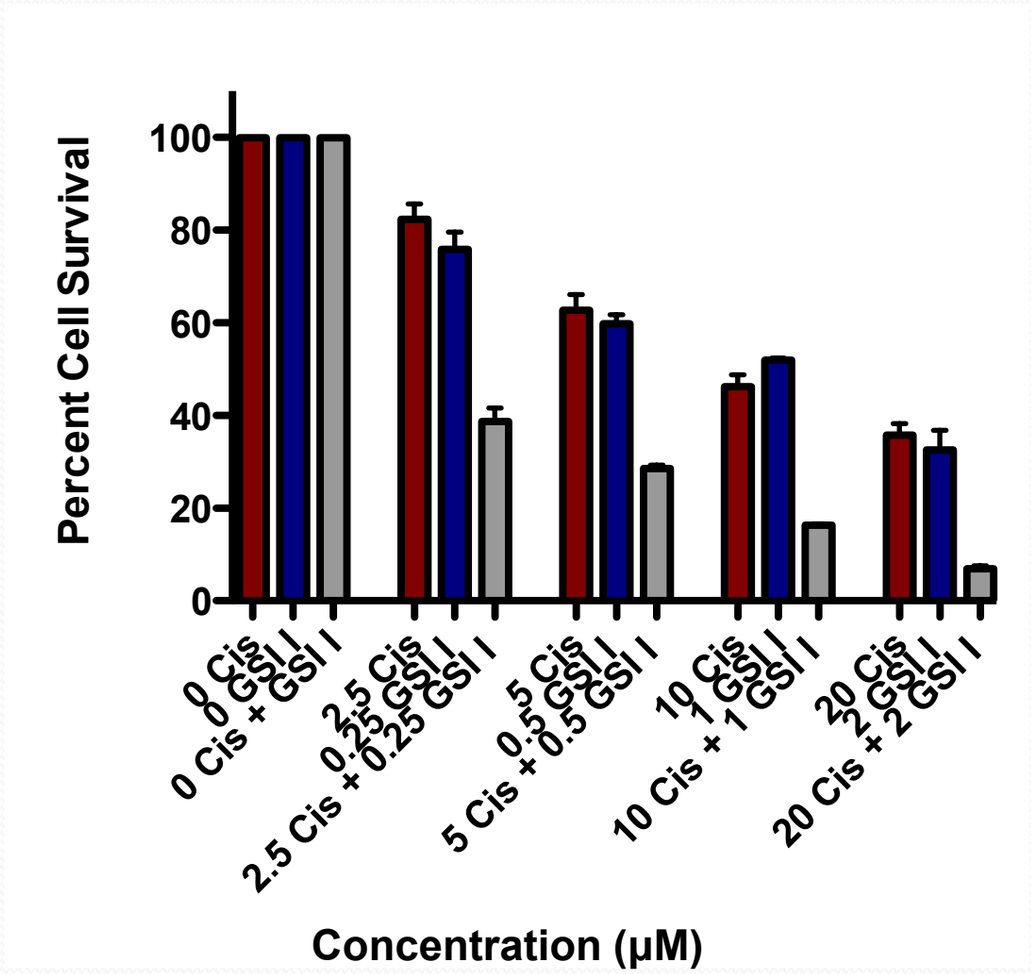


Blue: Nucleus staining

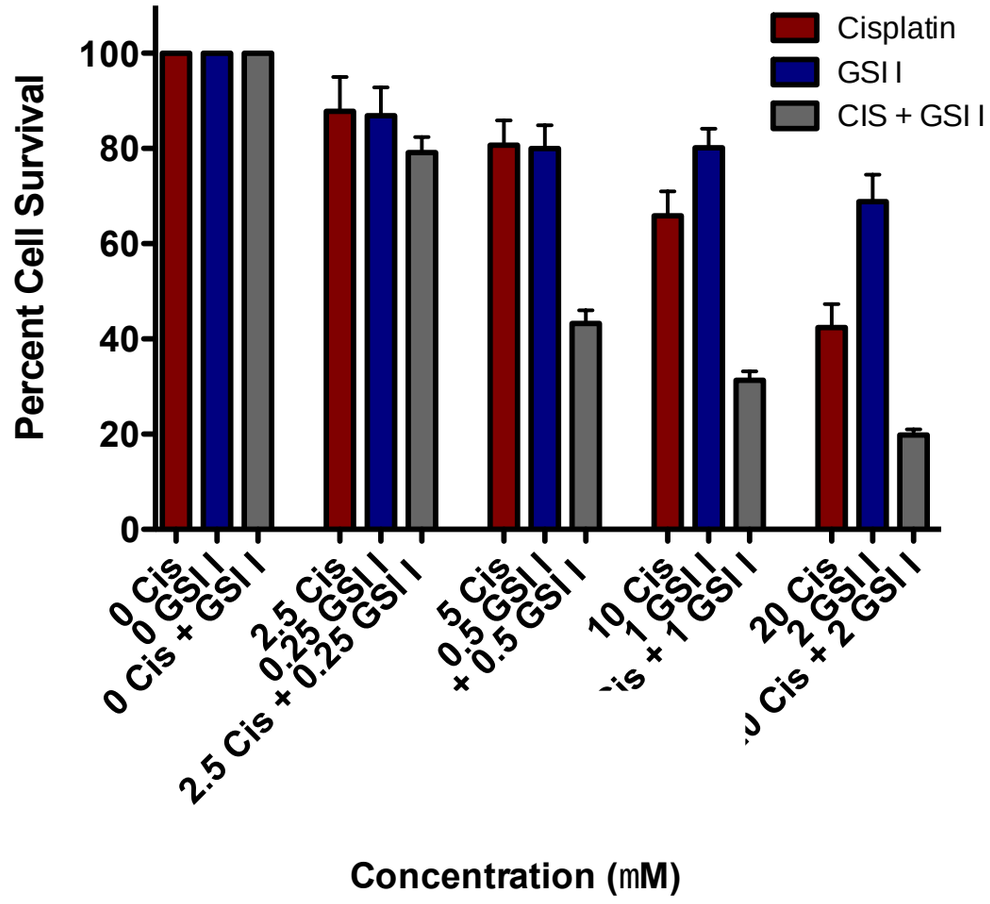
Red: Caspase 3 staining (apoptotic marker)

2D Results Confirmed by 3D Spheroid Models

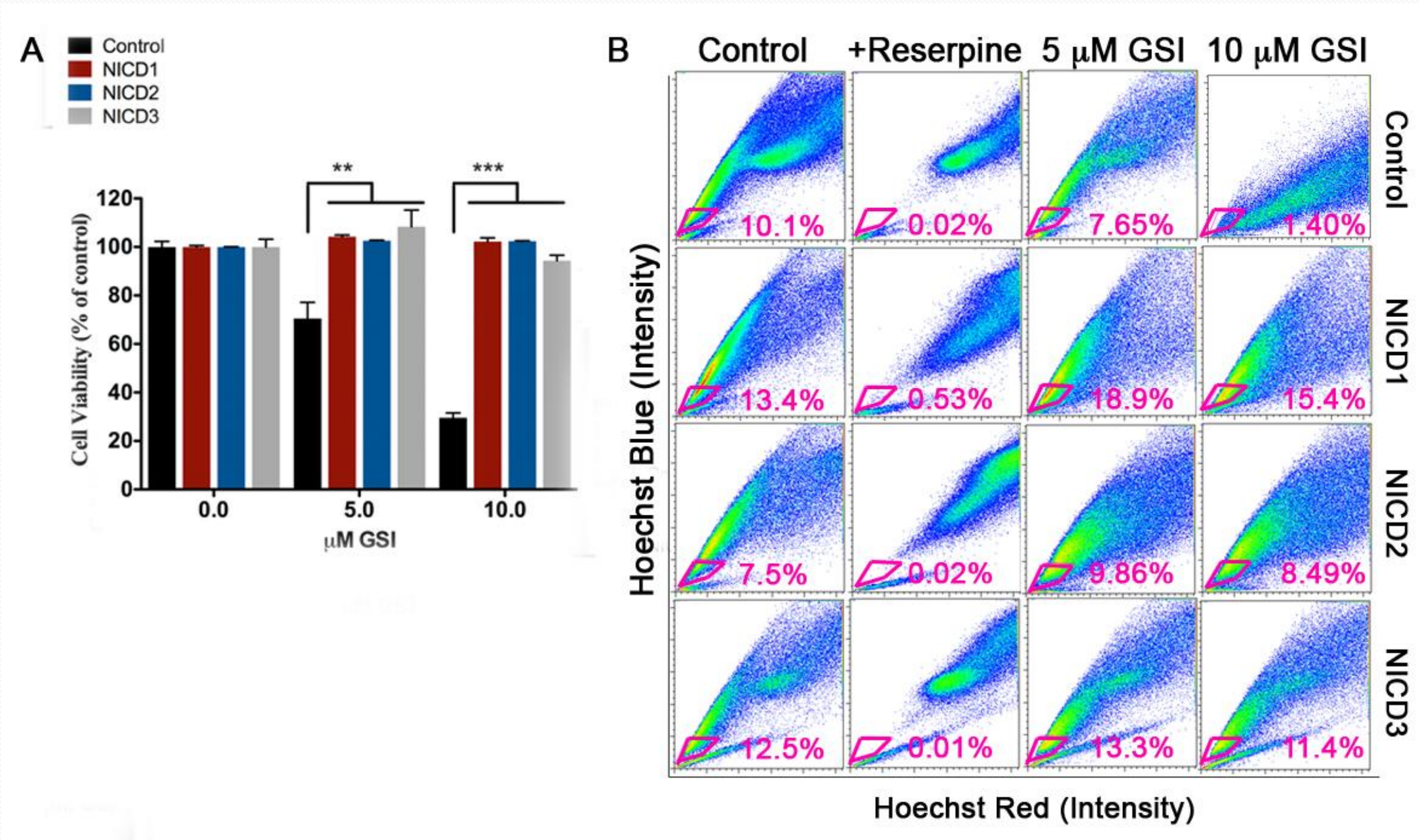
OVCAR5-2D



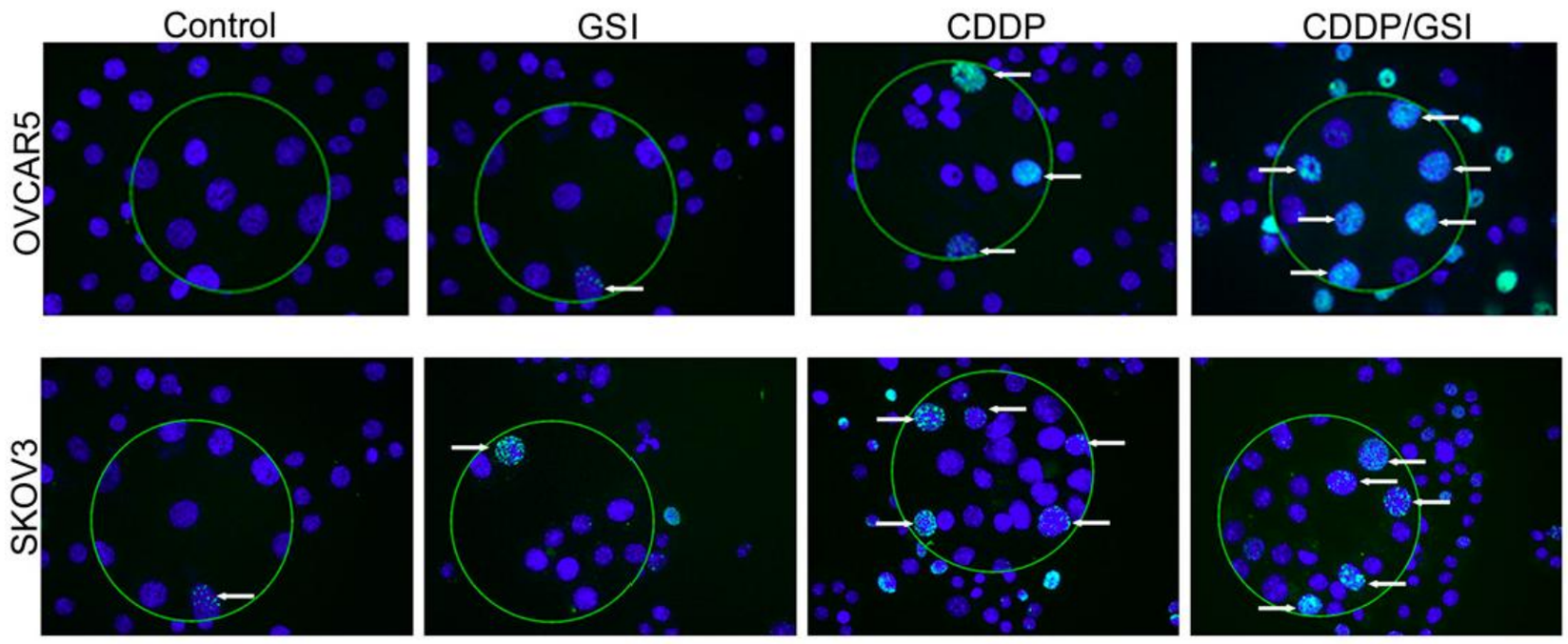
OVCAR5 Spheroids-3D



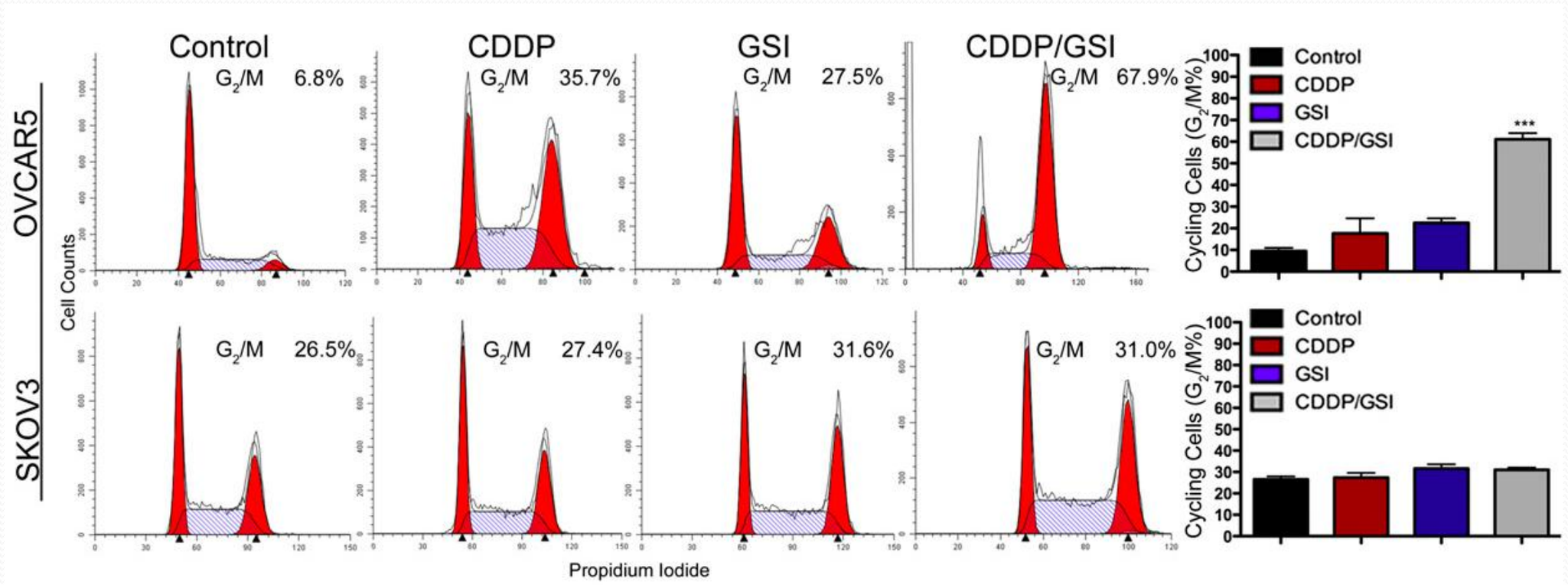
GSI Effects Are Notch Specific: Overexpression of Intracellular Notch ICD Rescues CSCs from GSI Treatment

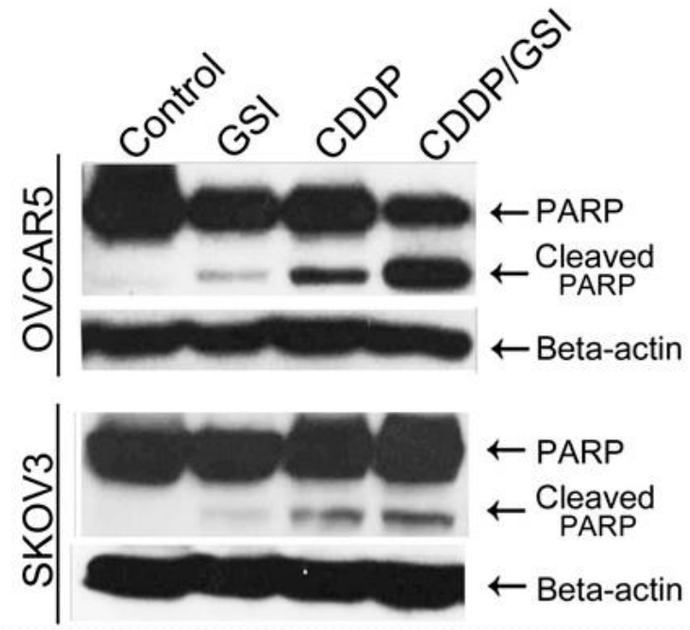


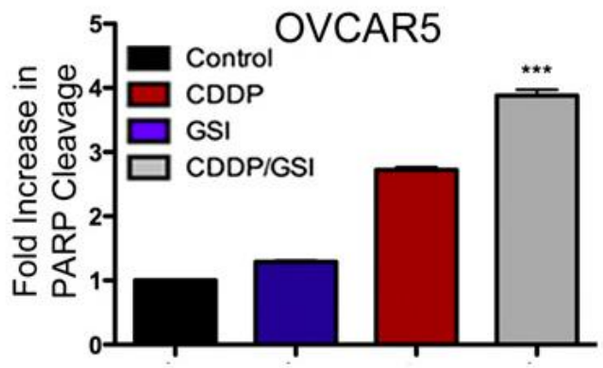
Platinum-GSI Co-therapy Is Effective Against Both CSCs and the Bulk of Tumor Cells

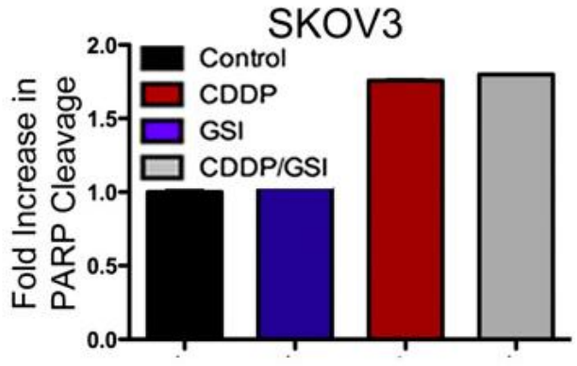


GSI and Platinum Co-therapy Shows Enhanced Response to DNA Damage and Cell Death in Notch-Dependent Tumor Cells

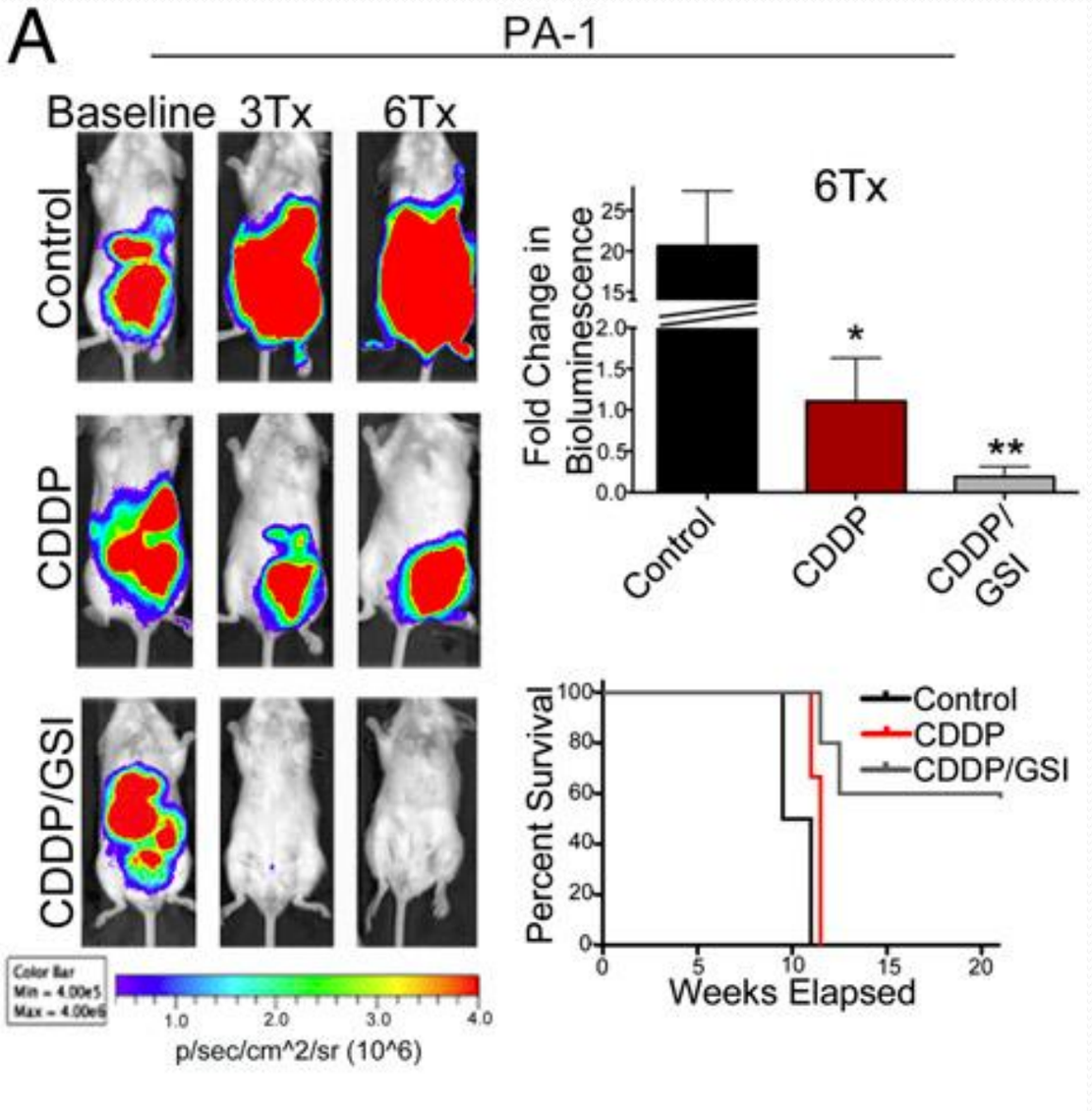


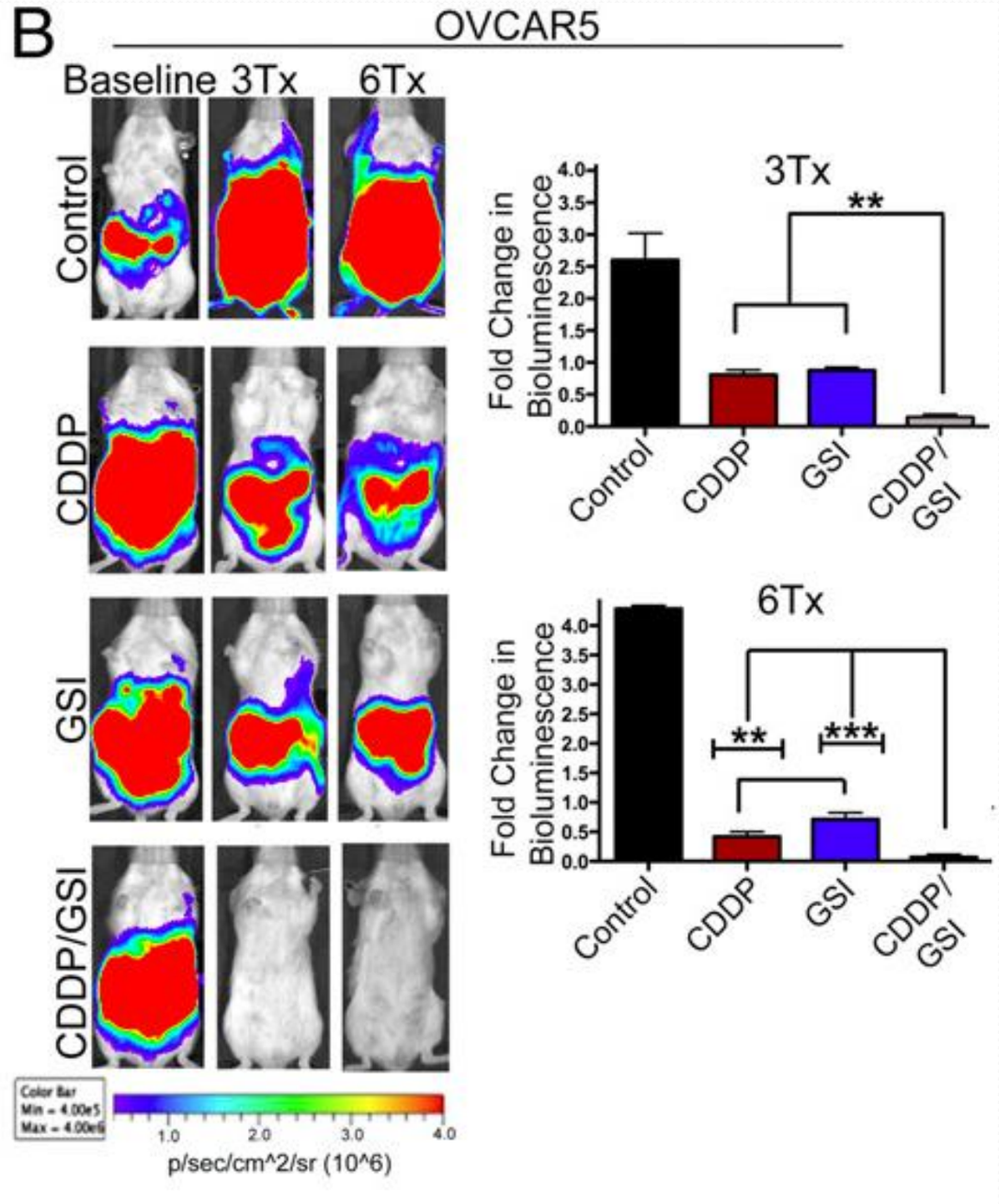




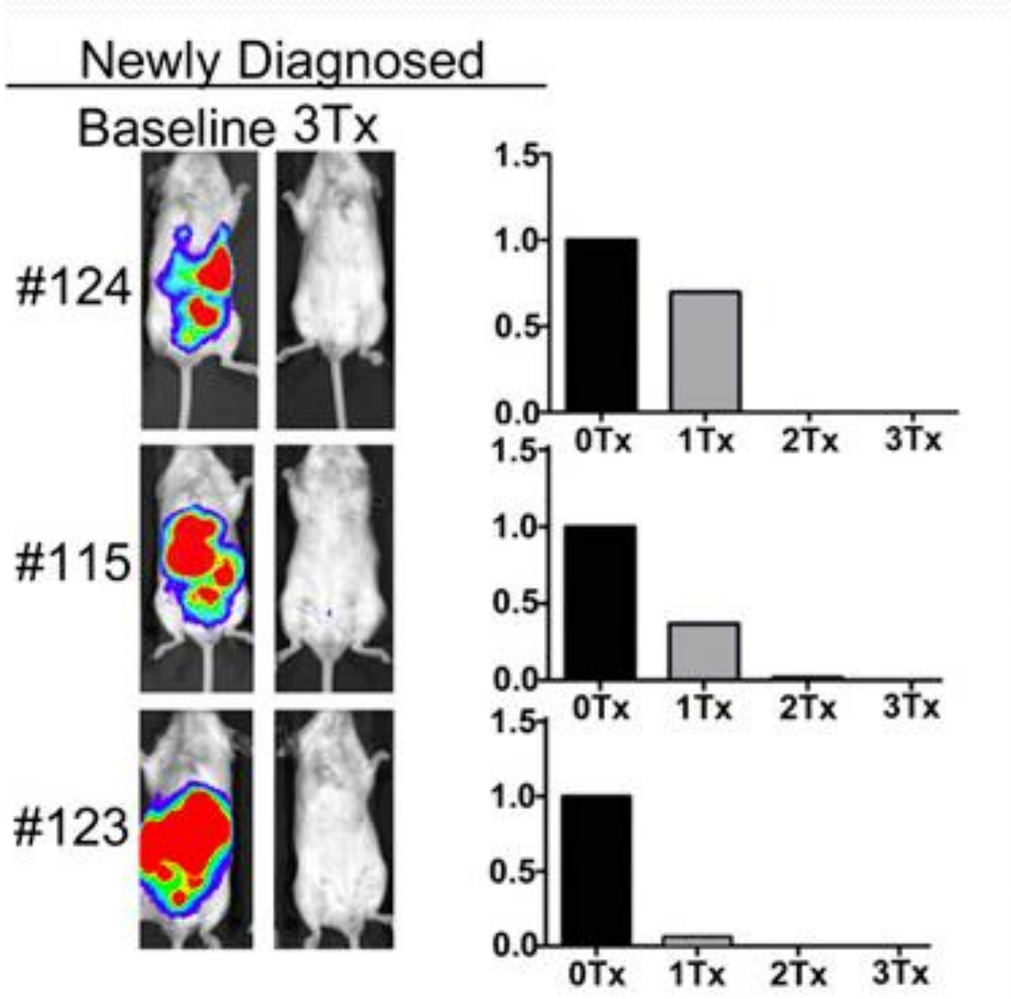


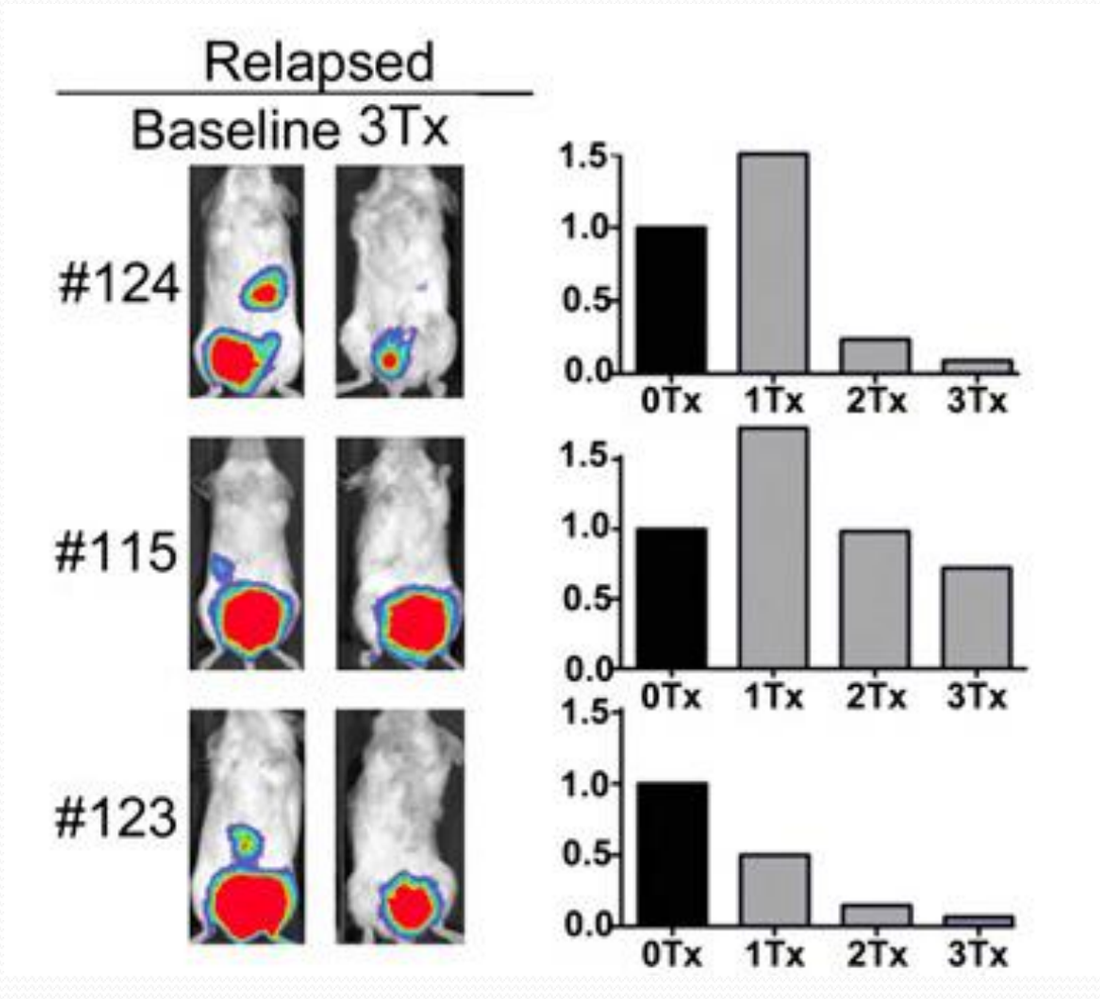
Notch Inhibitor Increases the Efficacy of Platinum Therapy and Survival in Tumor Xenografts with High Notch 3 Expression





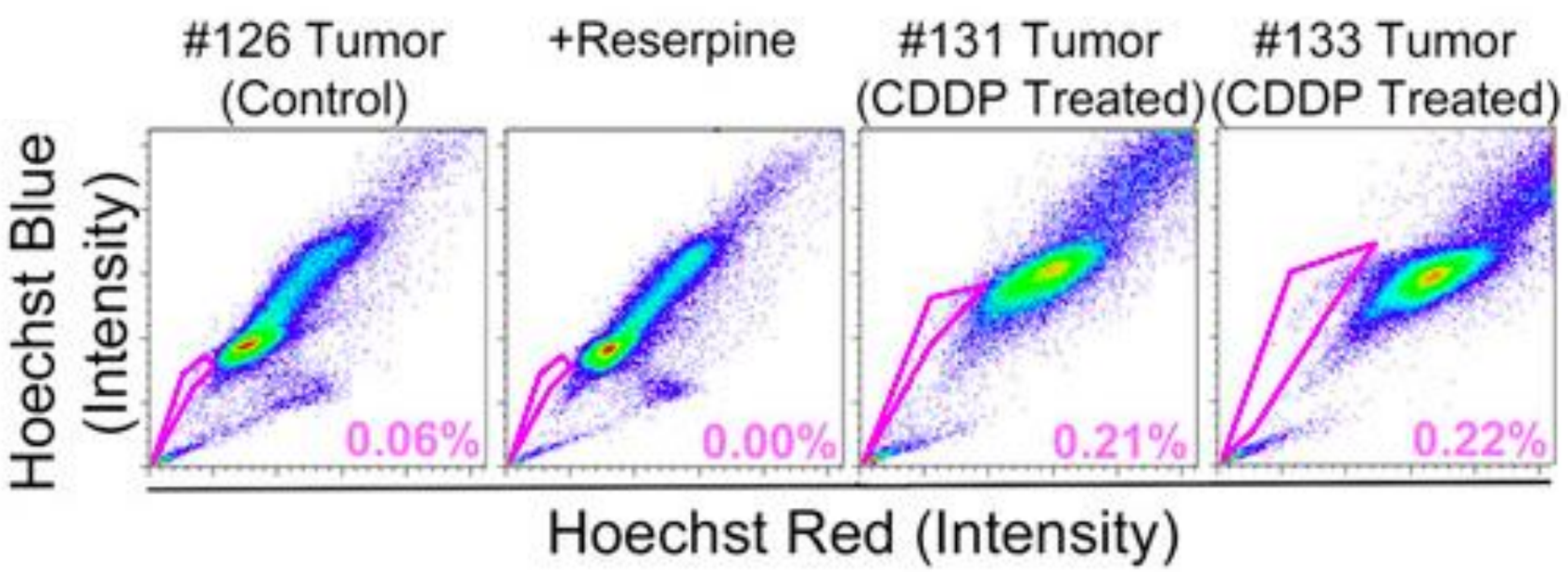
GSI I/CDDP Co-therapy in Relapsed Disease in Tumor Xenografts

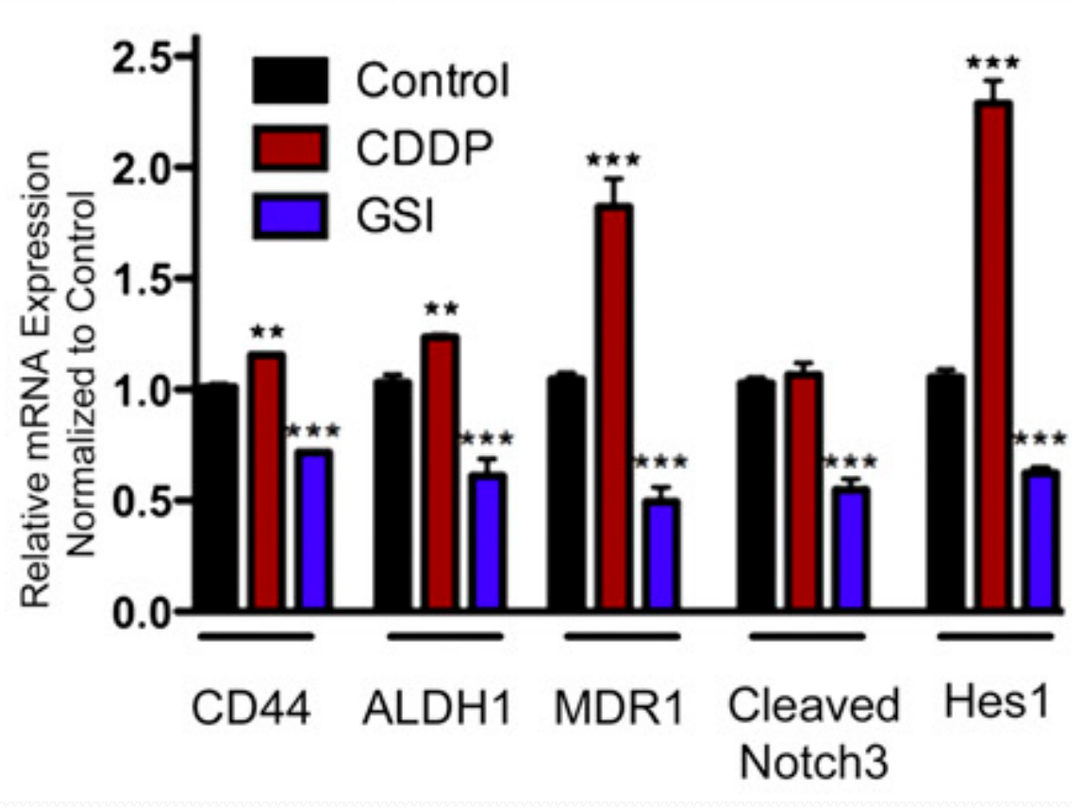




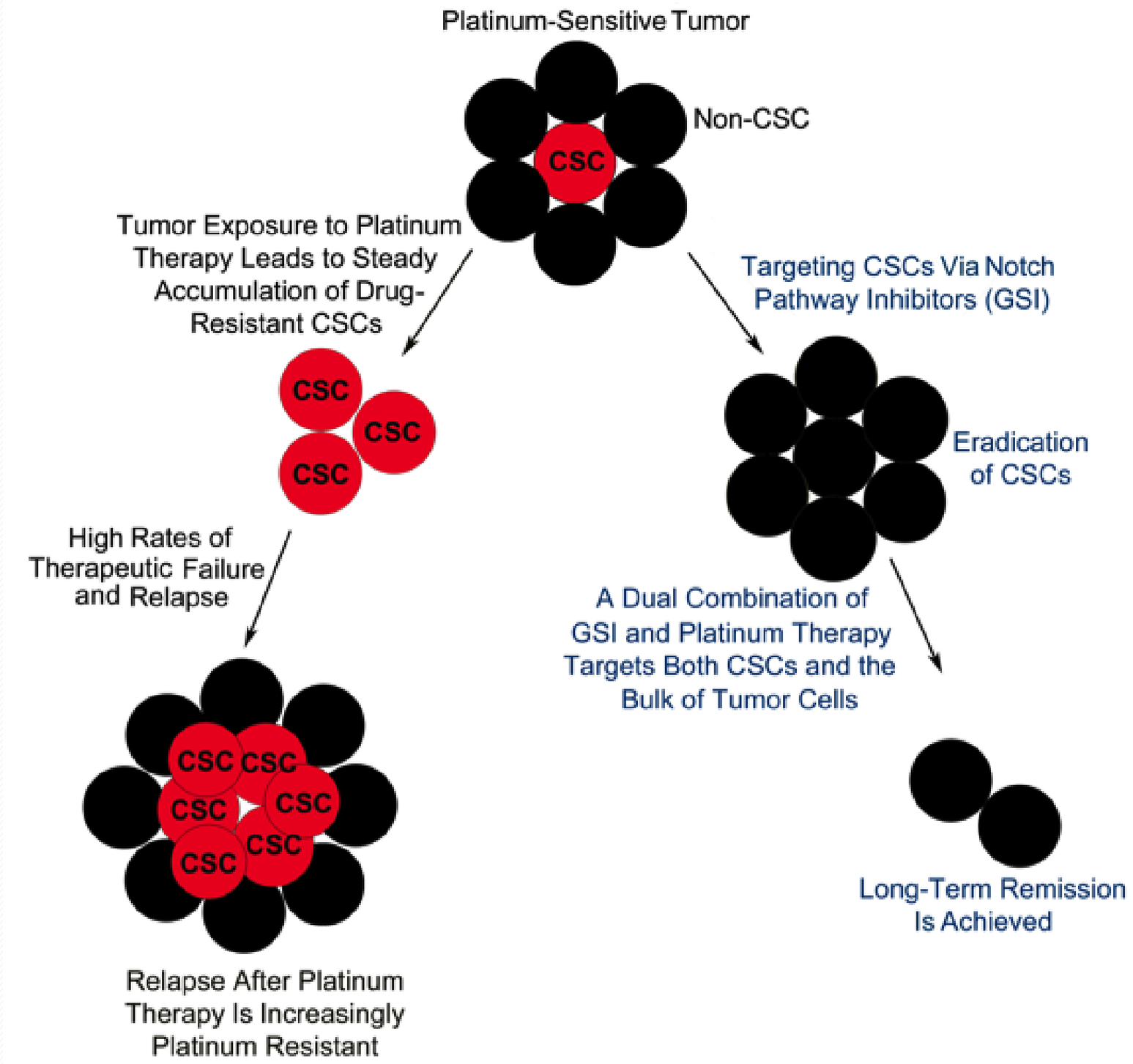


Platinum Treatment Enriches Relapsed Tumors for CSCs and Increases Stem Cell and Drug Resistance Markers





Implications for Novel Therapeutic Strategies



Summary Ovarian CSCs

1. We have identified and validated a functional and molecular profile of ovarian CSCs.

1. In addition to stem cell surface markers, such as CD44, ovarian CSCs express high levels of ABC multi-drug transporters (ABCG2, ABCB5, MDR1), which results in increased resistance to platinum and doxorubicin chemotherapy.

2. Notch plays a key role in the maintenance of the CSC niche in ovarian cancer cells. GSI, a Notch pathway inhibitor, increases sensitivity to platinum in both newly diagnosed and resistant patient lines.

3. Targeting Notch signaling pathway in cancer stem cells could help overcome the challenge of chemoresistance to platinum therapy in ovarian cancer.

Clinical Trials Targeting CSCs

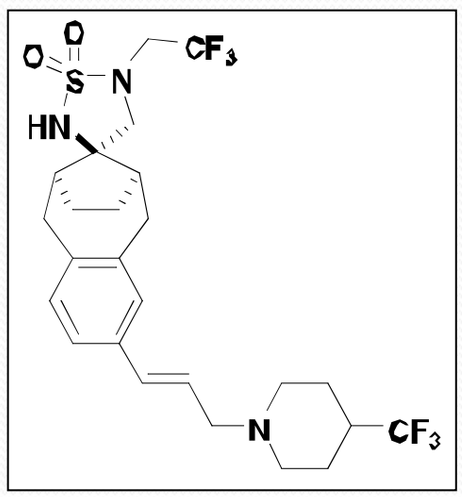
Gamma-Secretase Inhibitors

•**Roche compound** (RO4929097) tested in multiple trials of solid tumors. Compound failed in phase I, therapeutically effective doses caused CYP3A4 activation and auto-induction and no escalation could be achieved in patients.

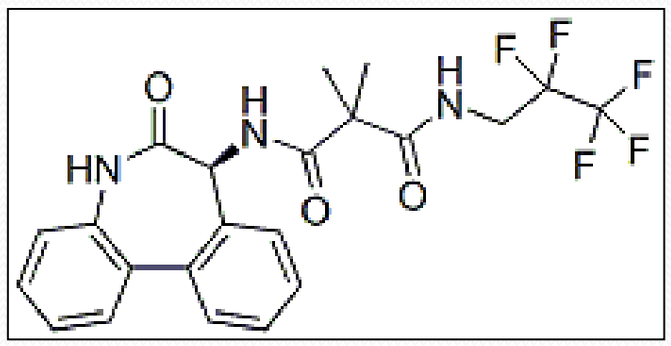
•**Merck** (MK-0752) promising, high efficacy but toxic when given orally due to inhibition of normal stem cells in the GI tract. Toxicity may be prevented if given i.p.

•There are over 20 different GSIs that differ greatly in structure and efficacy and need to be investigated.

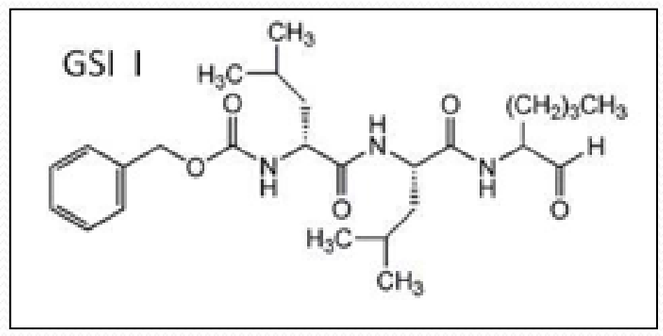
Merck 003



Roche Compound

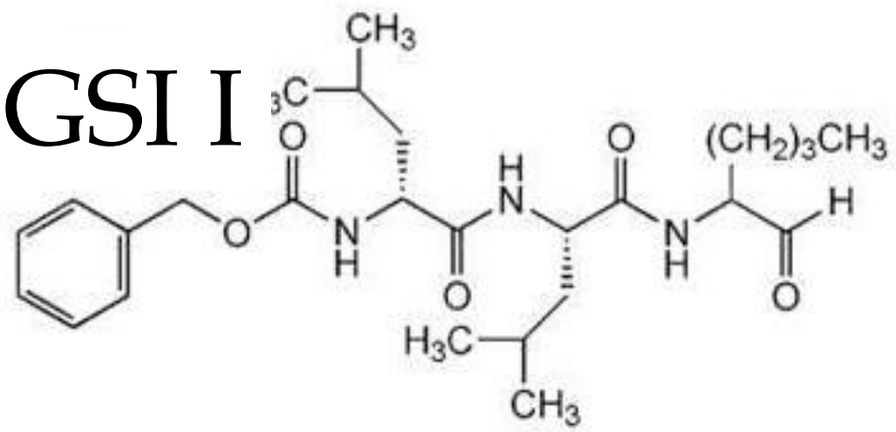


GSI I-Preclinical compound

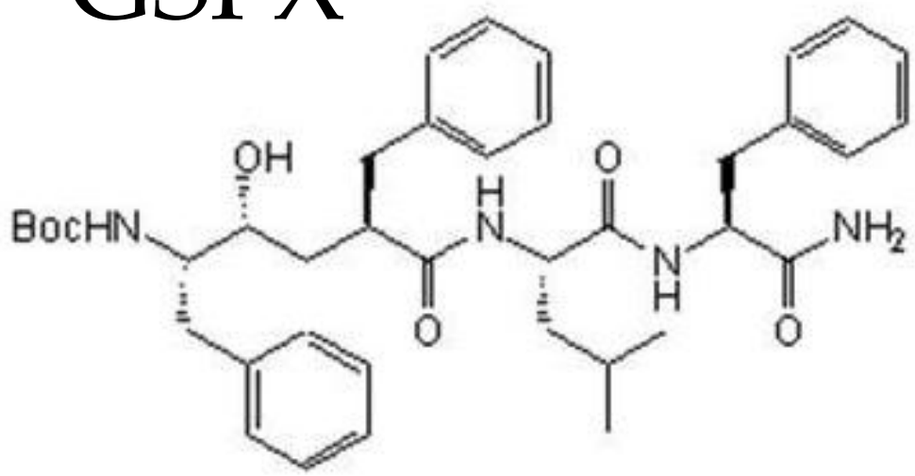


Chemical Structures of the Five Most Commonly Used Preclinical GSIs

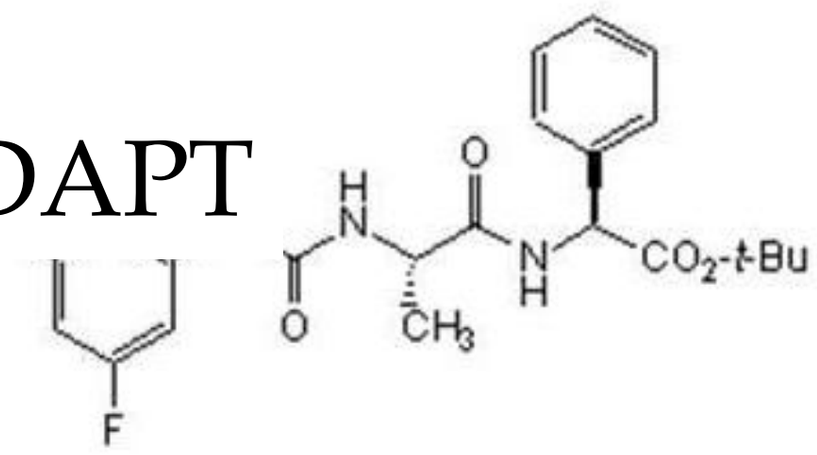
GSI I



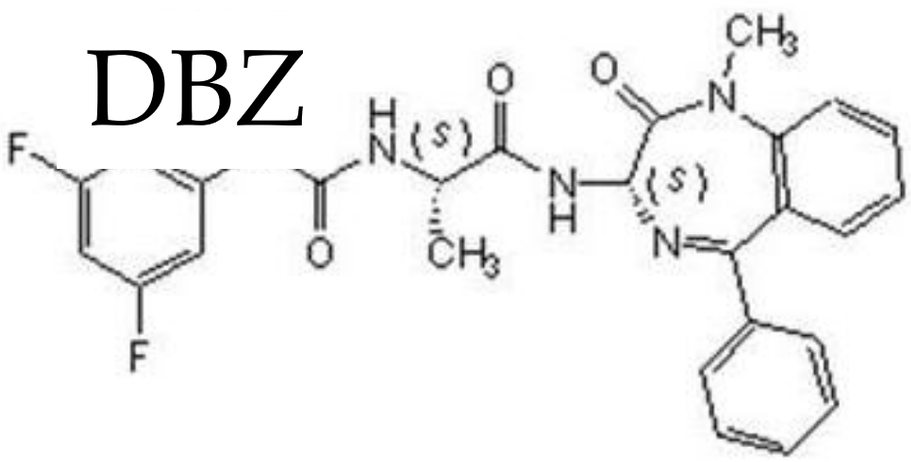
GSI X



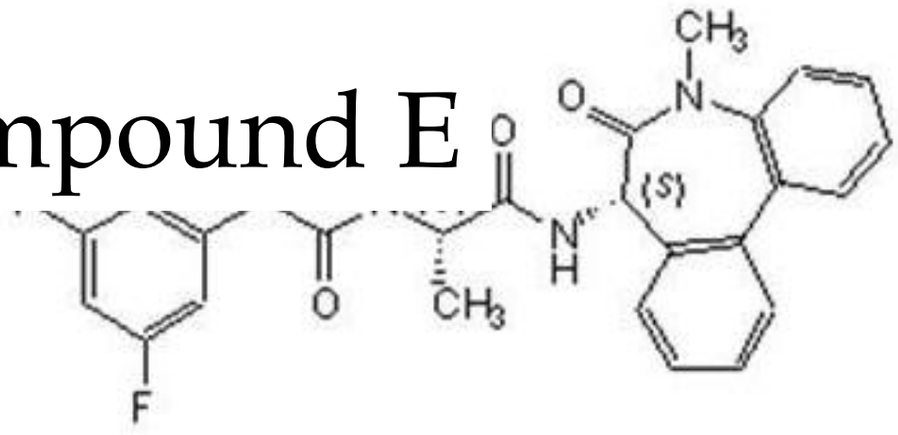
DAPT



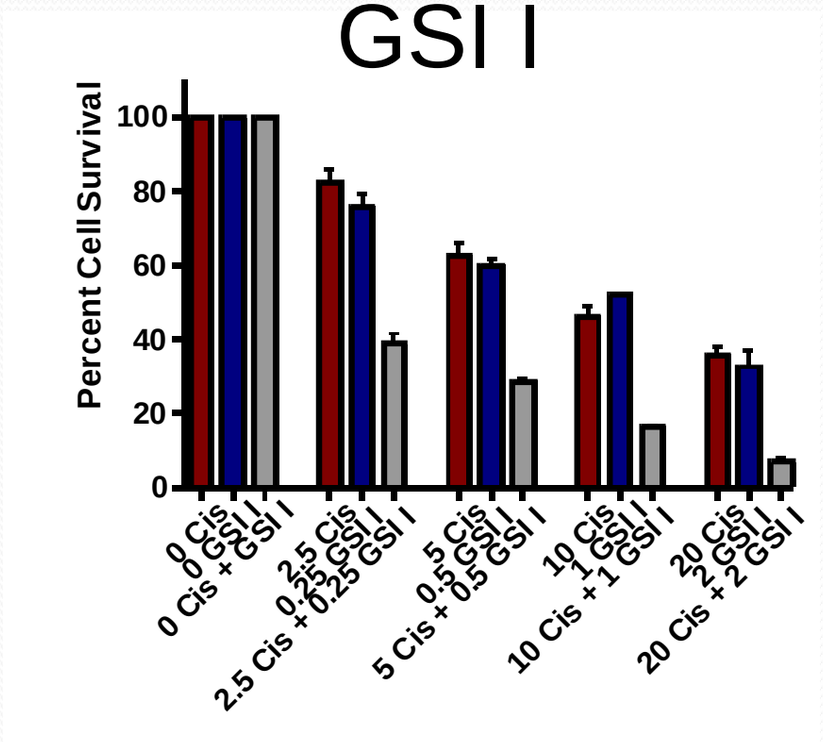
DBZ

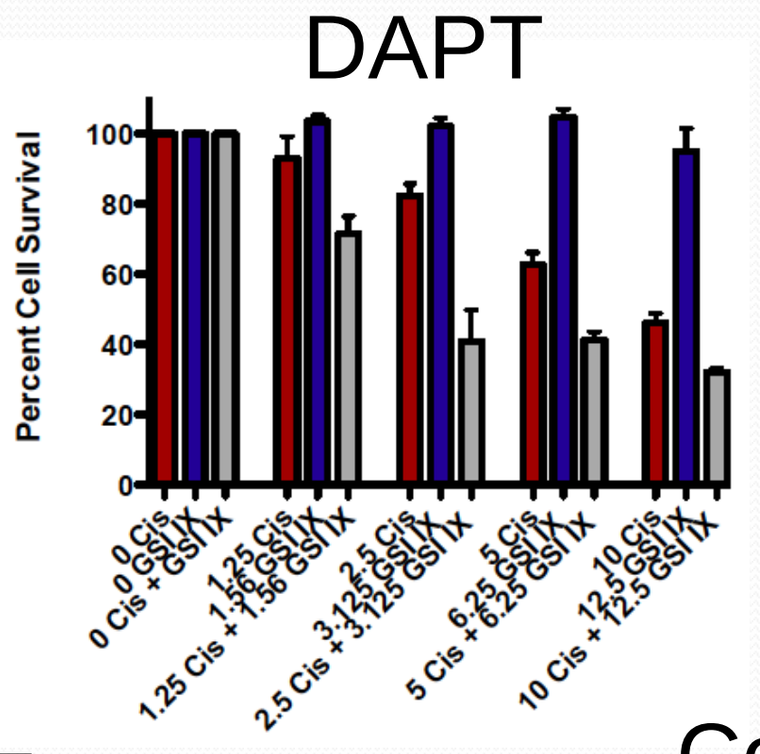


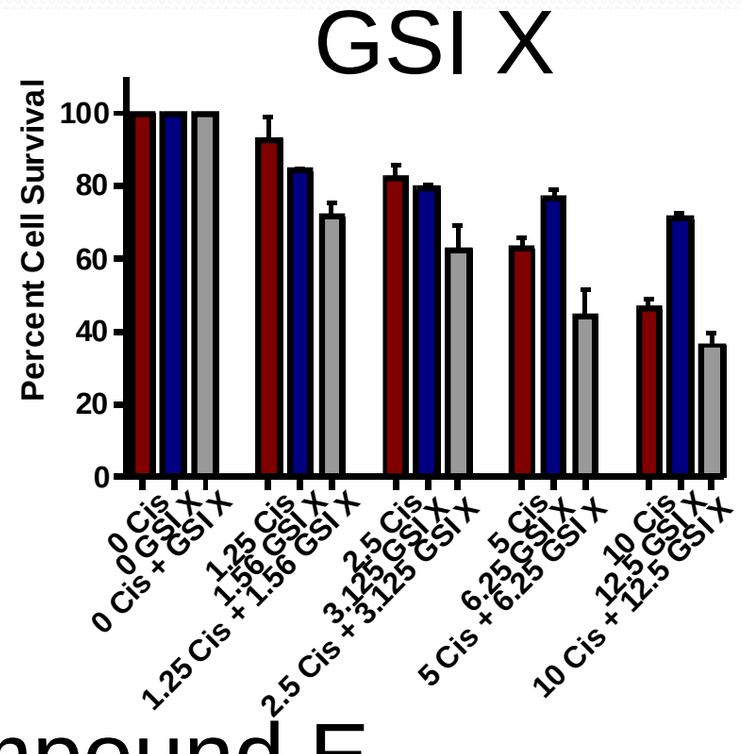
Compound E

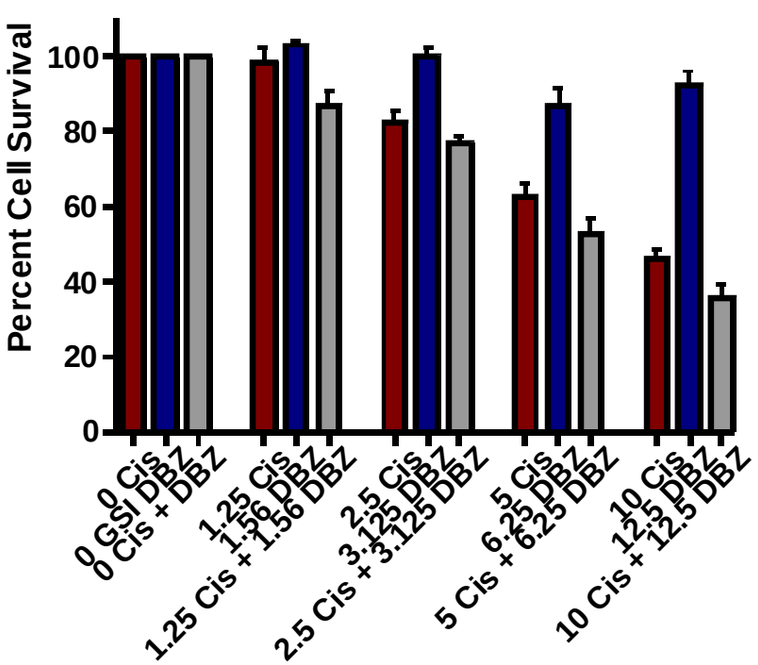


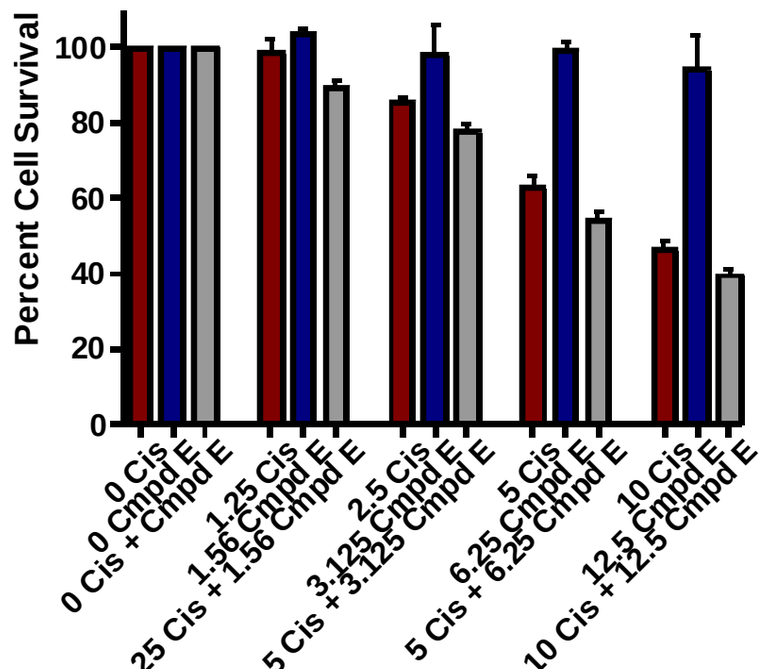
Synergistic Effect of CDDP/GSI Co-therapy is Preserved Independent of GSI Type





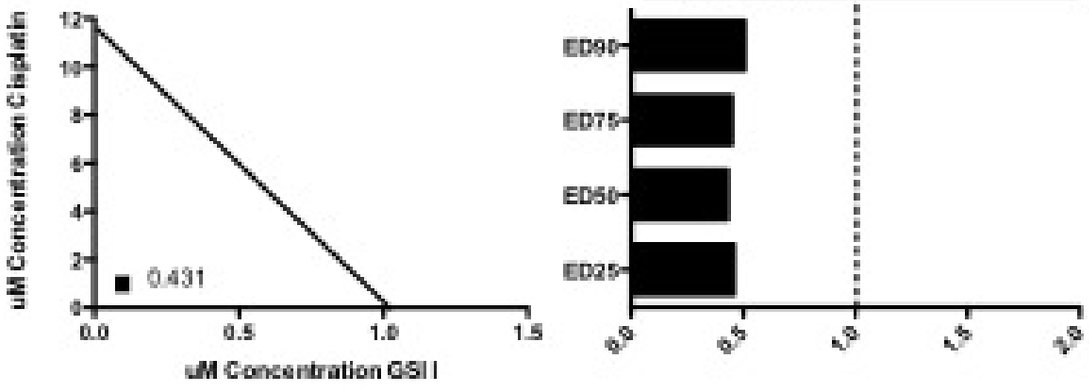




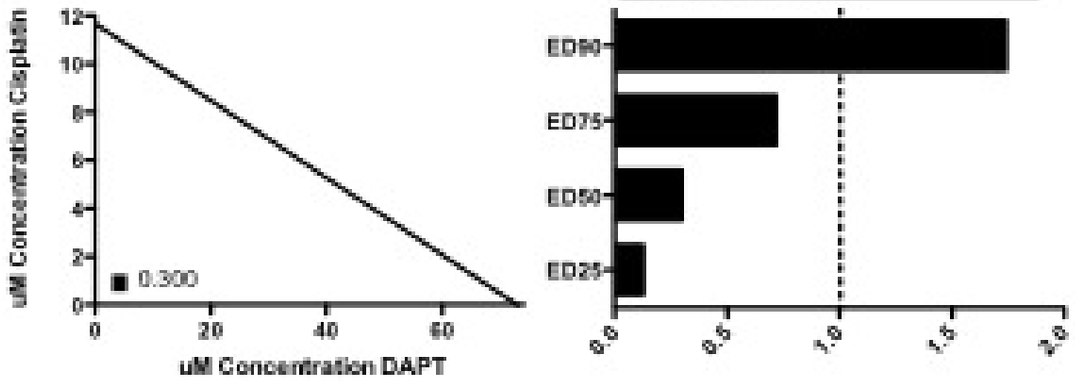


Synergistic Effect of CDDP/GSI Cotherapy is Preserved Independent of GSI Type (Isobologram Analysis)

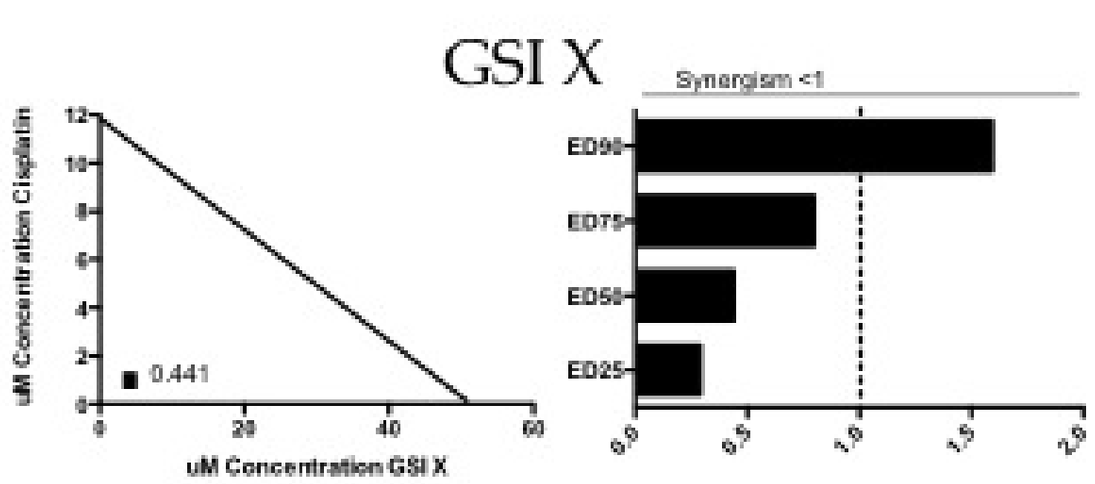
GSI I



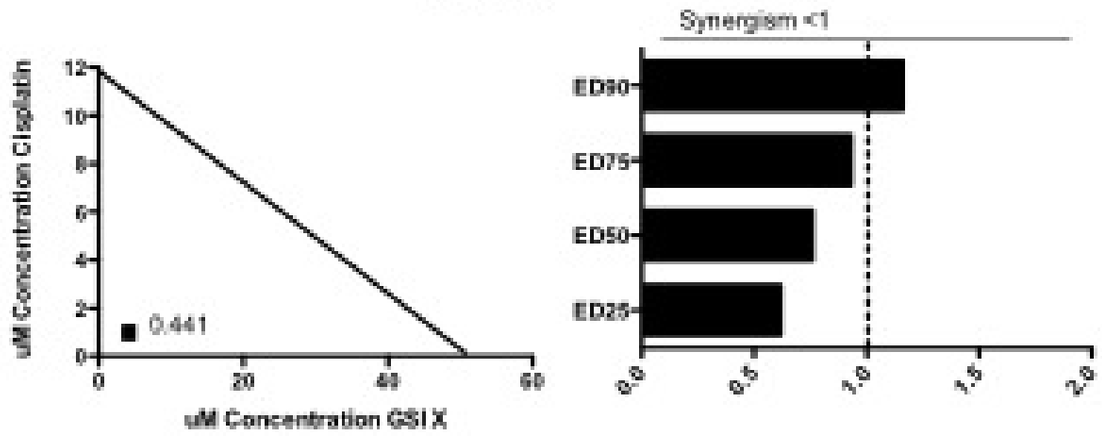
DAPT



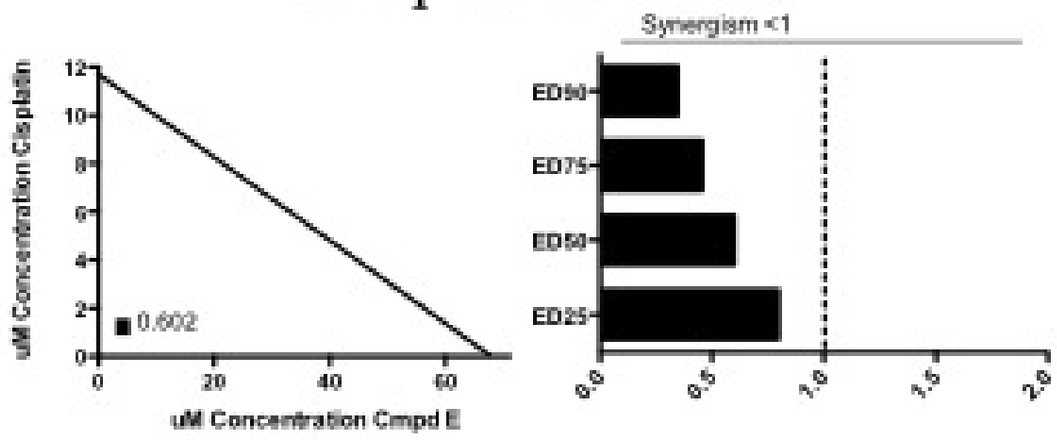
GSI X



DBZ



Compound E

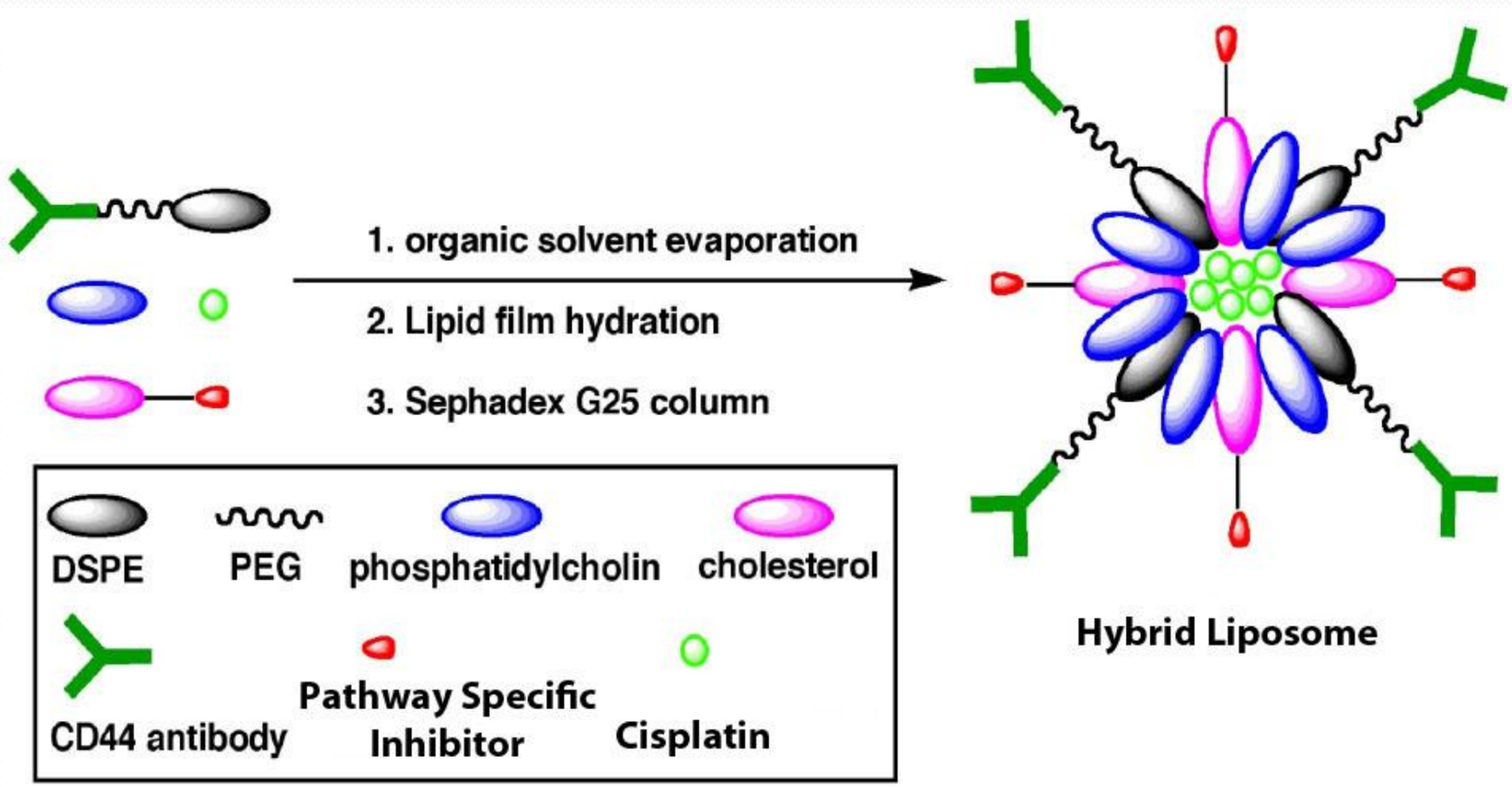


Conclusions

- GSI/CDDP co-therapy is more effective than currently used platinum therapies alone in eliminating cancer cells
- Better understanding of GSIs is critical to bringing this promising co-therapy to patients
  - Can we model drugs for clinical trials after preclinical GSIs?
- Additional methods of GSI delivery to evade toxicity concerns warrant further investigation

Nanoparticle-based Therapy Targeting CSCs

- Nanoparticles are engineered to deliver the “payload” of drug to the tumor cells only while healthy cells remain unaffected.
- Nanoparticles carrying drugs remain in circulation for much longer periods of time as compared to the drugs alone, which are clinically used today.
- They allow delivery of multiple drugs at the same time with increased efficacy and less toxicity.



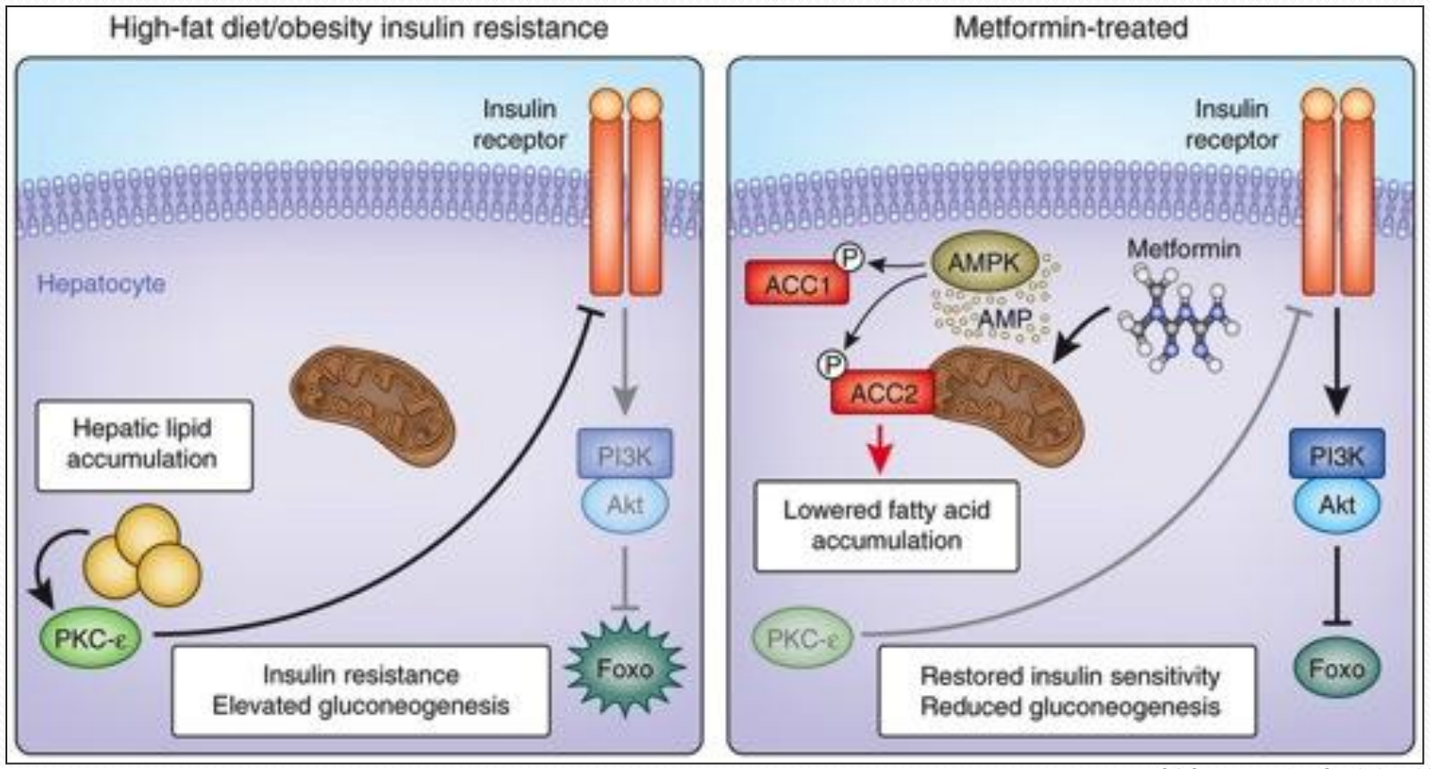
Use of Metformin in Clinical Trials Targeting CSCs

Metformin

•Utilized as a type II diabetes medication. Its potency in ovarian cancer was discovered when it was reported that diabetic patients with ovarian cancer had better outcomes (Shank et al 2012).

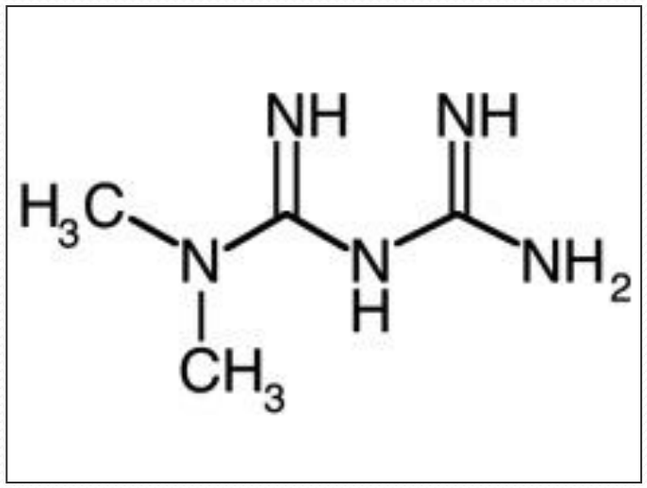
•Highly potent compound, which can reduce $ALDH^+$ CSC numbers to control levels (Shank et al 2012).

•NCT01579812: Targeting CSCs to prevent relapse in GYN tumors.



(Shaw et al. 2013)

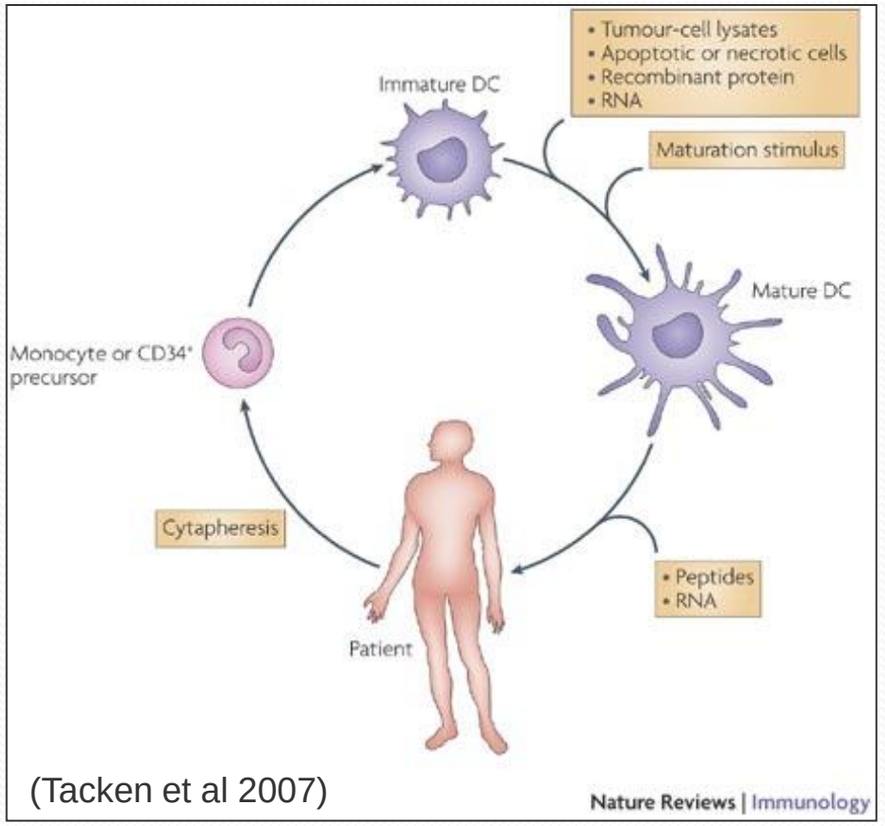
Metformin



Clinical Trials Using Vaccines to Target CSCs

Immunovaccines

- Multiple clinical trials of solid tumors based on Ning et al. 2012 study.



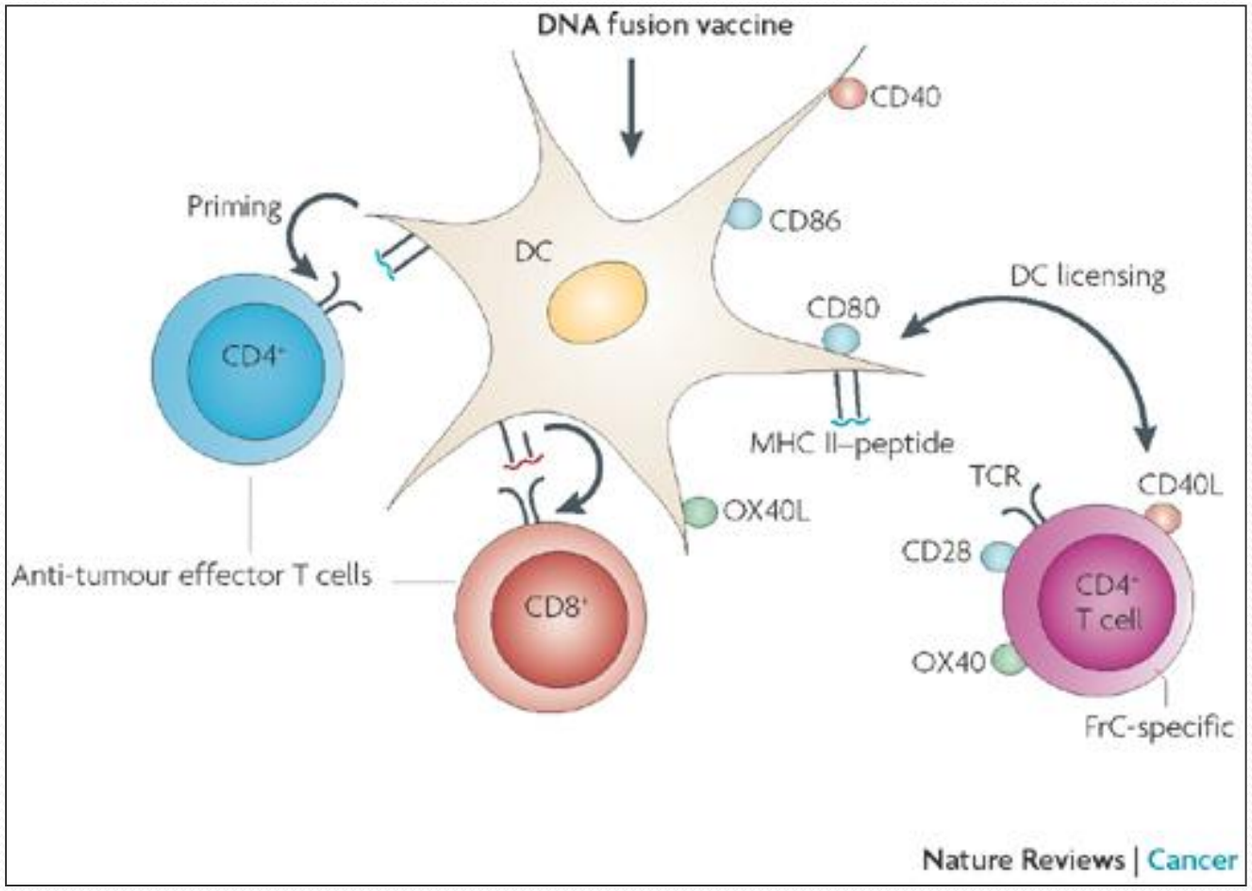
(Tacken et al 2007)

DC-vaccine	Clinical Trial	DC Vaccine components
Hepatocellular	NCT02089919	Includes isolating ALDH^{high} CSCs and creating a DC vaccine targeting CSCs (as described by Ning et al.)
Lung	NCT02084823	
Nasopharyngeal	NCT02115958	
Pancreatic	NCT02074046	

Clinical Trials Using Immuno-vaccines to Target CSCs

- Multiple clinical trials of solid tumors, including ovarian, based on study by Vik-Mo et al. (2013).

mRNA tumor CSCs	Clinical Trial	DC Vaccine components
Ovarian	NCT01334047	hTERT, survivin mRNA, amplified CSC mRNA
Glioblastoma	NCT00846456	mRNA isolated from CSCs



(Rice et al. 2008)

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